The effect of wheat cultivars on the growth performance and energy retention of broiler chickens

Thesis

THE EFFECT OF WHEAT CULTIVARS ON THE GROWTH PERFORMANCE AND ENERGY RETENTION OF BROILER CHICKENS

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ABSTRACT

The nutritive values of twelve samples of different UK wheat cultivars from two different growing years were assessed. A series of chicken feeding experiments were conducted to examine the relationships between chemical composition, grain quality and energy content of the wheat samples and the growth performance of broiler chickens when fed these wheat samples as part of nutritionally complete diets. The efficiency of utilization of apparent metabolisable energy (AMEn) of wheat samples as a source of net energy (NE) was studied.

Step-wise multiple regression analysis indicated that the content of total starch in the wheat samples and the amylose:amylopectin ratio in the starch were the main predictors of growth, feed intake and FCR of the broilers. Increasing starch and amylose content in the wheat cultivar samples gave increasing weight and feed intakes. The Hagberg falling number was also significantly ($p<0.05$) positively related to broiler chicken growth and food intakes. Two further experiments indicated that there was a growth response to increasing the amylose:amylopectin ratio, but that the response was specific only to the variations in amylose:amylopectin ratio by using an extracted starch from a high amylose maize cultivar. Variation of the amylose:amylopectin ratio by using different rice cultivars gave no ($p>0.05$) differences in broiler growth performance. The influence of different amylose:amylopectin ratios on the physical nature of the starch granule may therefore be more important than their effects in changing the total dietary supply of those starch components.

Although the net energy concentration of a wheat sample was related ($p<0.05$, $r^2=0.42$) to its determined AME, there was still unexplained variation (about 60%) in the efficiency of utilization of AME as a source of NE. A proportion of this variation was, however, explained ($p<0.05$, $r^2=0.40$) by differences in the water-extract viscosity of the wheat samples. Different ileal viscosities, due to feeding different wheat cultivars, may result in variation in the amount of intestinal fermentation of nutrients and so alter the NE/ME ratio of wheat-based diets.

DECLARATION

This thesis was composed by the author and is a record of work carried out by him on an original line of research. All sources of information are shown in the text and listed in the references; all help given by others is indicated in the acknowledgements.

None of this work has been presented in any previous application for a degree.

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This thesis is dedicated to my parents.

PUBLISHED WORK

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Pirgozliev, V. R. & Rose, S. P. (1999). Net energy systems for poultry feeds: a quantitative review, *World's Poultry Science Journal* **55:** 23-36.

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LIST OF ABBREVIATIONS

AM	Amylose
AM:AP	Amylose to amylopectin ratio
AME	Apparent metabolisable energy
AMEn	Apparent metabolisable energy corrected for retained nitrogen.
AP	Amylopectin
DF	Degrees of freedom
DM	Dry matter
FCR	Feed conversion ratio
HFN	Hagberg falling number
ME	Metabolisable energy
NE	Net energy
NSP	Non-starch polysaccharide
SEM	Standard error of the mean
TME	True metabolisable energy
TMEn	True metabolisable energy corrected for retained nitrogen.
EEL	Endogenous energy losses

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1. INTRODUCTION

1.1. GENERAL INTRODUCTION

Wheat is an important source of energy in UK poultry feeds and can comprise up to 80% of the diet for finishing broilers (Longstaff & McNab, 1986; Wiseman & Inborr, 1990). The energy concentration in wheat, per unit of price, makes it economically competitive in diet formulations. Wheat typically contains about 60-70% starch and can supply about 70% of the available energy content of a broiler chicken feed. Wheat also can supply up to 40% of the amino acid requirements of poultry (McNab, 1996). About 15 million tonnes of wheat were produced in the UK in the 1997/98 harvest year and more than 30% of it was used as animal feed (HGCA, 1998). Animal feed compounders were the largest single user of cereals in the country.

More than one million tonnes of meat from broilers are produced in the UK alone each year (MAFF, 1998). Wheat is often the only cereal used in British broiler feed formulations, so its nutritional value and variations in its feeding quality are very important commercially. Variation in the chemical composition (Bolton & Blair, 1974; Aman, 1988), metabolisable energy (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Annison, 1991) and broiler growth performance (March & Biely, 1973; Rose *et al.*, 1993; Waldron, 1997) have been reported for different wheat samples. Only a few studies have attempted to explain the variation in growth performance from the determined chemical composition of the wheat samples. These studies (March & Biely, 1973; Waldron, 1997) have used food formulations in which only the samples of wheat were varied. The diet formulations supplied a small excess of protein,

essential amino acids, minerals and vitamins, so that any possible nutrient deficiencies due to variations between wheat samples were avoided. None of the variations in proximate nutrient composition were related to the differences in growth performance in a large study of Canadian wheat samples (March & Biely, 1973). Waldron (1997) identified that a laboratory technique to measure the *in vitro* rate of starch digestion was connected to differences in growth performance between two wheat cultivars and Rose *et al.* (1993) identified a relation between Hagberg falling number and feed conversion ratio in six wheat cultivars. There is a need to further examine the relationship between chemical composition of different wheat cultivars and broiler chicken growth performance. In particular, there is a need to examine, in detail, the carbohydrate composition of different wheat cultivar samples and relate these to the differences in nutritional value for broiler chickens.

Metabolizable energy (ME) is a convenient measure of available energy in poultry diets and so most published studies have used ME as the major variable to describe the nutritional value of wheat samples for broilers. However, there is no consistent relationship between ME and the chemical composition of UK wheat samples (McNab, 1991; Wiseman & McNab, 1995; Waldron, 1997; Wiseman *et al.*, 1998). Also, differences in broiler productive performance, when fed different wheat samples, do not correlate with the determined ME of the wheat samples (Rose & Bedford, 1995; Waldron, 1997).

The lack of relationship between ME and growth performance is unexpected. The explanation could be due to variation between wheat samples in the efficiency of utilization of the ME. The work of Collier *et al.* (1996) and Blears *et al.* (1997) showed no difference in determined ME in UK wheat samples but different carcass

energy retentions. There is a lack of information about the efficiency of utilization of ME of different wheat samples and their relation to growth performance. Similarly, there is a need to examine how the chemical composition of wheat samples relates to any differences in the efficiency of utilization of ME as a source of net energy.

This project had two major objectives:

(i) To relate the differences in the chemical composition of twelve separate wheat cultivar samples to differences in growth performance (growth, feed intake and feed conversion ratio) of broiler chickens when fed these wheat samples as part of nutritionally complete diets. Particular emphasis was placed on examining the differences in the polysaccharide content of the wheat samples.

(ii) To examine whether there were differences between different wheat cultivar samples in the efficiency of utilization of ME as a source of NE. Then to examine the relationship between differences in chemical composition of the wheat sample and any differences in the efficiency of utilization of ME.

These objectives were achieved by examining two groups of samples, each of six separate wheat cultivars, from two growing seasons. Animal-based experiments measured growth performance in 7-21 d old broilers, AMEn in growing broiler chickens, NE by a comparative slaughter technique in broiler chickens and TMEn in adult cockerels.

The large amount of information produced by this work resulted in time only being available to further examine the results and conclusions from objective 1. Two further broiler chickens experiments were therefore conducted to examine whether the (relatively small) differences in total starch and amylose:amylopectin in the total diet due to feeding different wheat cultivar samples were a cause of differences in growth performance of growing broiler chickens.

The next three chapters (1.2, 1.3 and 1.4) review the literature concerning the chemical composition of wheat, wheat grain structure and availability of nutrients of wheat. Chapter 1.4 compares different systems of metabolisable energy measurement and a quantitative review of systems of net energy evaluation.

1. 2. CHEMICAL COMPOSITION OF WHEAT

Wheat is widely used in UK broiler chicken diets and many other countries and it is known that its nutritive value is variable. In general, the nutritive value of any feedstuff is influenced by its chemical composition and the degree to which the birds are able to digest, absorb and utilise these components. The purpose of this section is to compare the chemical composition of different wheats and to make a comparison of some of these variables with other cereals.

The presented information that is not specifically referenced in the text has been derived from the following sources: Seed Physiology of Development and Germination (Bewley & Black, 1994); Seed Biology (Kozlowski, 1972); Economic Botany (Simpson & Conner-Ogorzaly, 1986); Wheat and Wheat Improvement (Heyne, 1987); Toxic Constituents of Plant Foodstuffs (Liener, 1980); The New Oxford Book of Food Plants (Vaughan & Geissler, 1997); Animal Nutrition (McDonald, Edwards & Greenhalgh, 1994) or Osnovi na Hraneneto (Todorov, Marinov & Alexiev, 1995).

1. 2. 1. Wheat grain structure

The wheat grain has a similar physical structure to the other cereals, and can be divided into three major parts, bran layer, endosperm and germ (or embryo). A mature wheat seed consists of about 13% bran layer, 85% endosperm and 2% embryo.

A generalised diagram of the structure of whole wheat grain and its cross section are given in Figure 1.1.

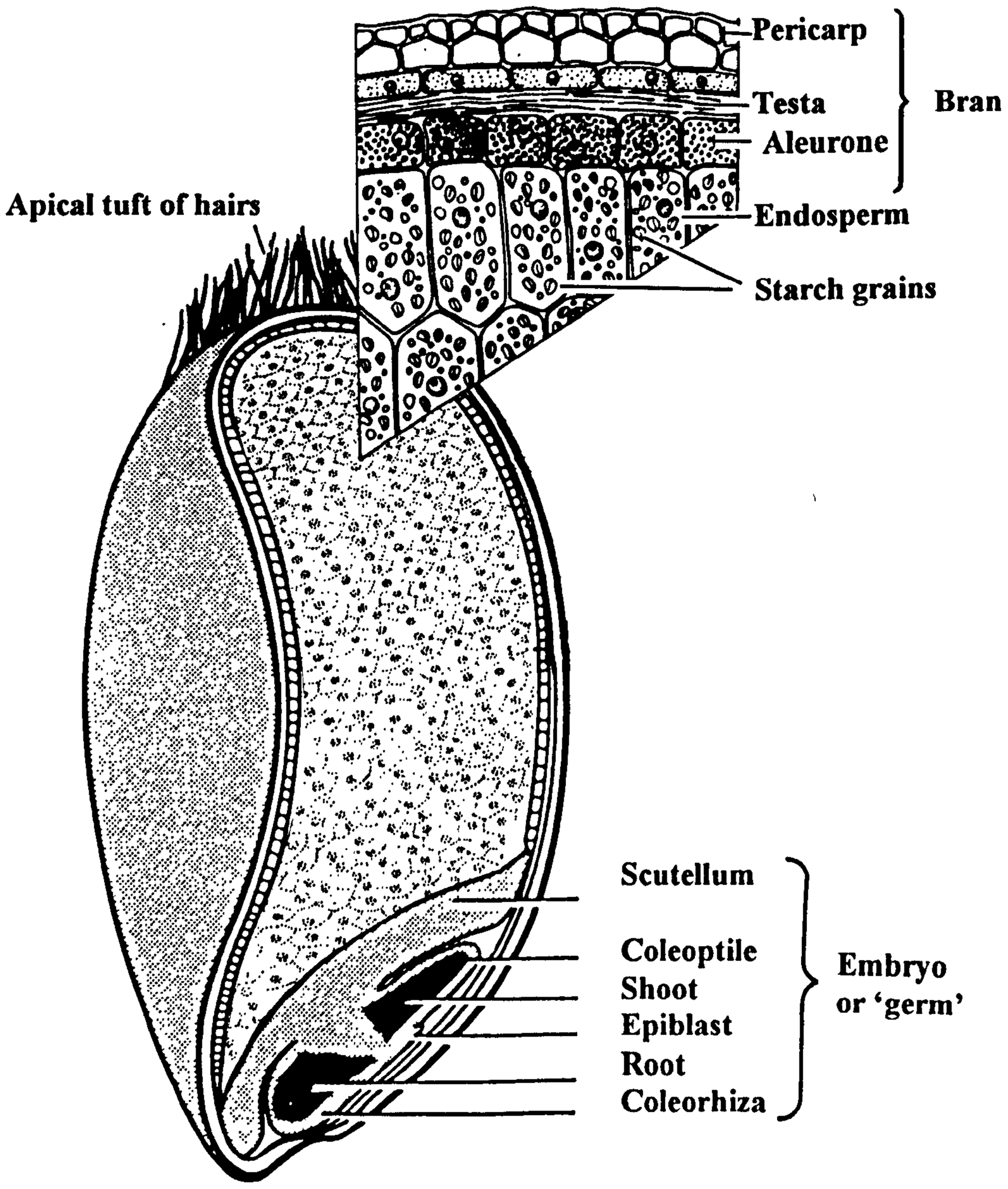
(i). Bran

The bran comprises pericarp, testa (seed coat) and aleurone layers and they can be distinguished only microscopically. The pericarp and testa are the outside layers and aleurone is the inside layer of the bran. The bran is particularly rich in dietary fibre/non-starch polysaccharides (NSP), the vitamin B complex, and minerals.

The seed coat is a structure of considerable importance because it forms the barrier between the embryo and its immediate environment. A most important property of the seed coat is its permeability to water and sometimes to gases. Impermeable seed coats confer seed dormancy. As long as water entry is blocked, no germination can occur. The coloring and texture of seed coats are distinguishing features of many seeds but sometimes cannot be used taxonomically because they may change as a result of environmental and genetic influences during development (such as polymorphism in seeds).

The aleurone layer contains less dietary fibre than the pericarp or the testa. The cells of this layer are typically rich in protein, fats and vitamins. The aleurone layer plays a key role in the germination of grain seeds because it secretes the enzyme (α-amylase), which breaks down the endosperm starch into sugars absorbed by the expanding embryo.

FIGURE 1. 1. WHEAT GRAIN STRUCTURE



Adapted from Simpson & Conner-Ogorlazy (1986) and McDonald *et al.* (1994)

(ii). Endosperm

Endosperm is the starchy tissue that forms during grain development. It provides energy for the developing embryo, and for the seedling after germination until it can establish itself. Endosperm comprises about 60-85% of the mature wheat grain. It contains a variety of storage materials such as starch, lipids, protein or hemicelluloses.

Cell walls of dead parenchymous core cells combine with the polysaccharides, β-glucan and arabinoxylan to form the honeycomb matrix of the endosperm. Starch granules which are stored in the cavities of the endosperm are generated by organelles called amyloplasts which are present in the cells of the developing seed.

(iii). Embryo (

The embryo is an immature new plant (sporophyte) that is arrested in a dormant state in the seed (Heyne, 1987). The scutellum is the highest part of the embryo, and during germination it secretes some hormones and regulates endosperm mobilization. The lower part of the embryo consists of the relatively massive primary root enclosed by the coleorhiza. The embryo itself contains high levels of proteins, fats, and vitamins. The embryo is usually separated from endosperm before flour milling because oil can become rancid

Certain cereal processes, such as milling, will affect the nutrient content of the products because of the unequal distribution of nutrients. The production of white flour from bread wheat grain is a good example. In this instance the object of the process is to remove the bran and germ, leaving the flour. This will remove much of the fibre, vitamins, and minerals, and consequently will affect the nutritional value of the flour.

1. 2. 2. Polysaccharide composition

Plant materials contain two main, and chemically distinct, types of polysaccharides: the storage polysaccharide, starch; and the cell-wall polysaccharide, that is predominantly not starch (non-starch polysaccharide) (NSP) (Englyst & Cumming, 1988). Starch is stored in seeds in two related forms, amylose and amylopectin. NSPs include cellulose (insoluble) which is the main component of plant cell walls, and non-cellulose polysaccharides (mainly water soluble), including: β-glucans, arabinoxylans, pectin, gums and mucilages.

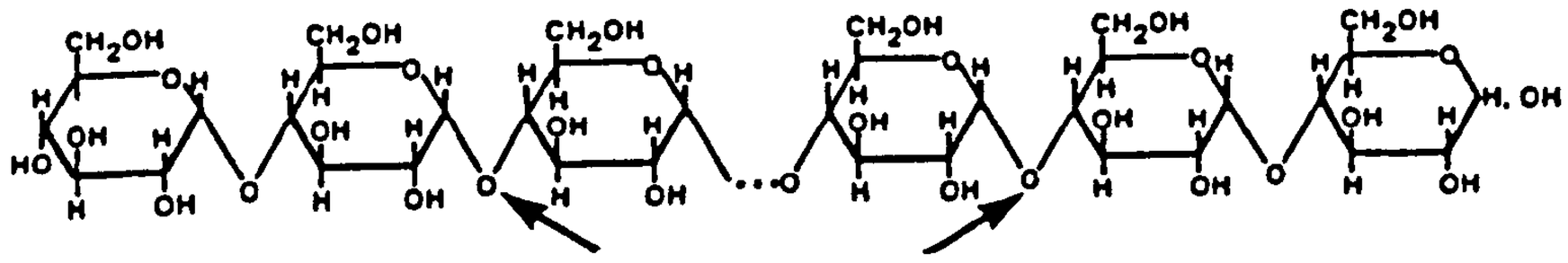
1. 2. 2. 1. Starch

Starch is the predominant reserve carbohydrate found in plants, and comprises between 64-74% of the endosperm in wheat grains. Starch content in wheat has been reported by different authors and there is some variation in these data that could be due to different analysis techniques: 504-596 g/kg (Rogel *et al.*, 1987), 532-724 g/kg (Nicol *et al.*, 1993), 604-660 g/kg (Waldron, 1997).

Cereal endosperm starch has a granular form and it is composed of amylose and amylopectin. Amylose consists of D-glucosyl units joined by α (1→4) linkages to form straight chains (Figure 1. 2). It has an average molecular weight of $14x10^3$ g/mol. Amylose may also contain infrequent branches, but still essentially behaves as a linear polymer (Lineback & Rasper, 1988). Polymers in the range between 6500-160000 as synthesized by the plant are insoluble because they exist as a double helix (Kodama *et al.*, 1978). Each double helix has two amylose polymers twisted around one another in a right-handed sixfold turn that repeats every 20.8 Å. The interior of the helix is mostly composed of hydrogen atoms attached to carbon atoms and it results in a hydrophobic surface. The amylose that is found in nature is crystallized in either of two forms that differ from one another in packing density and amount of associated water (Figure 1. 3). The crystals from type A are more tightly packed and lack room for water to exist as compared to crystals from type B. The A-crystals are commonly found in grains, while the B-crystals are usually found in tuberous plants (Wu & Sarko, 1978). The amylose content of most wheat samples varies between 24-30% depending on the wheat cultivar and growing year (Evers *et al.*, 1974). Naturally occurring reduced amylose wheat mutants have recently been identified. Hybridization experiments have resulted in the development of amylose-free or 'waxy' and amylose-reduced or 'partial waxy' wheat cultivars. The genetics, properties and potential applications of such waxy wheats were reviewed by Graybosch (1998).

Amylopectin also contains α (1→4) D-glucosyl chains, but branches occur every 20-25 residues due to the presence of α (1→6) linkages (Figure 1. 4). It is heavier than amylose and its molecular weight is about $4x10^6$. Amylopectin has a

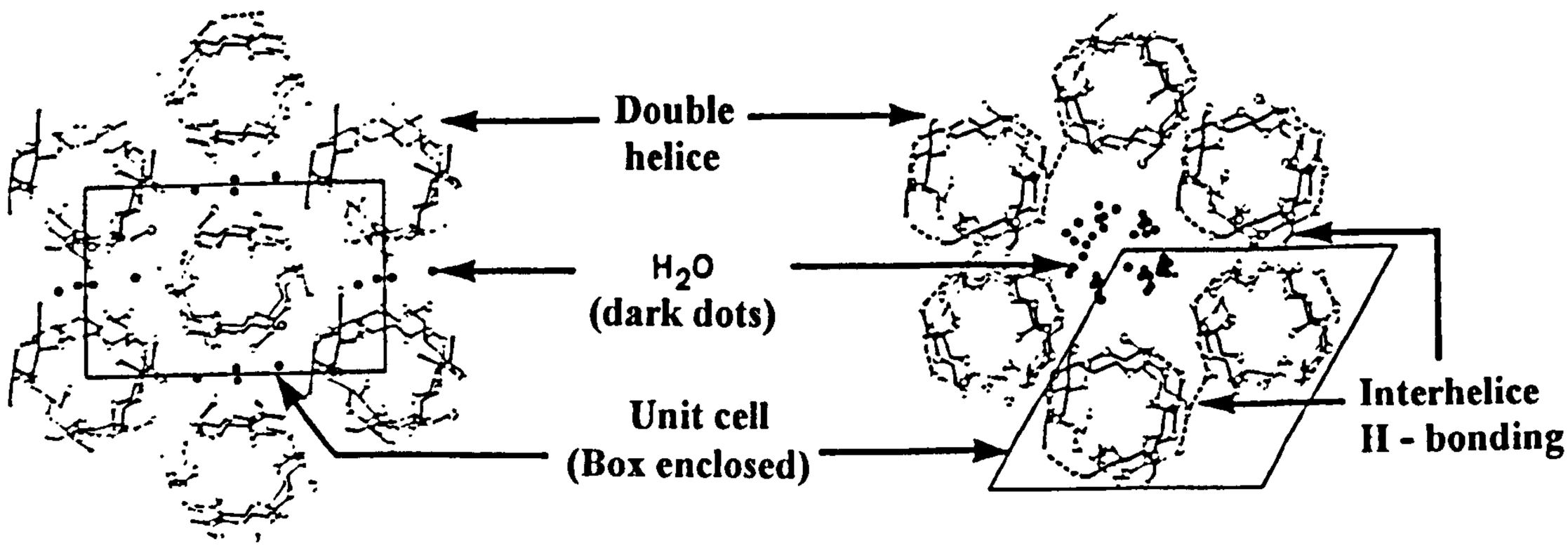
FIGURE 1. 2. AMYLOSE STRUCTURE.



Non-reducing end **α-1,4 linkages** **Reducing end**

Adapted from Moran (1982).

FIGURE 1. 3. AMYLOSE CRYSTALS.



'A' Amylose **'B' Amylose**

Adapted from Sarko & Wu (1978) and Moran (1982).

FIGURE 1. 4. AMYLOPECTIN STRUCTURE.

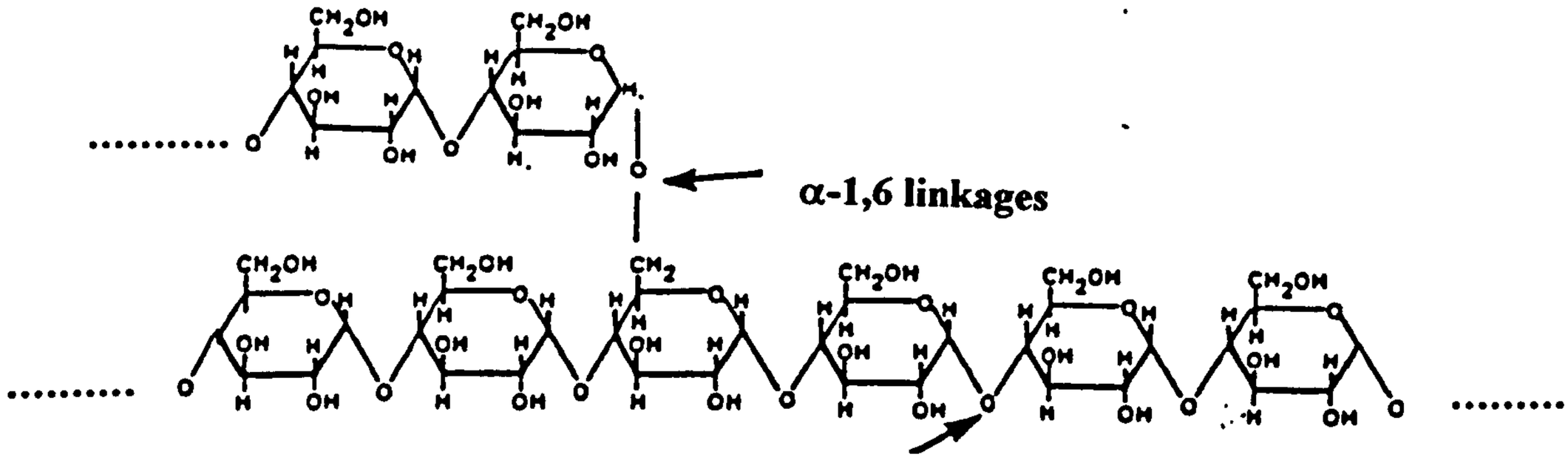
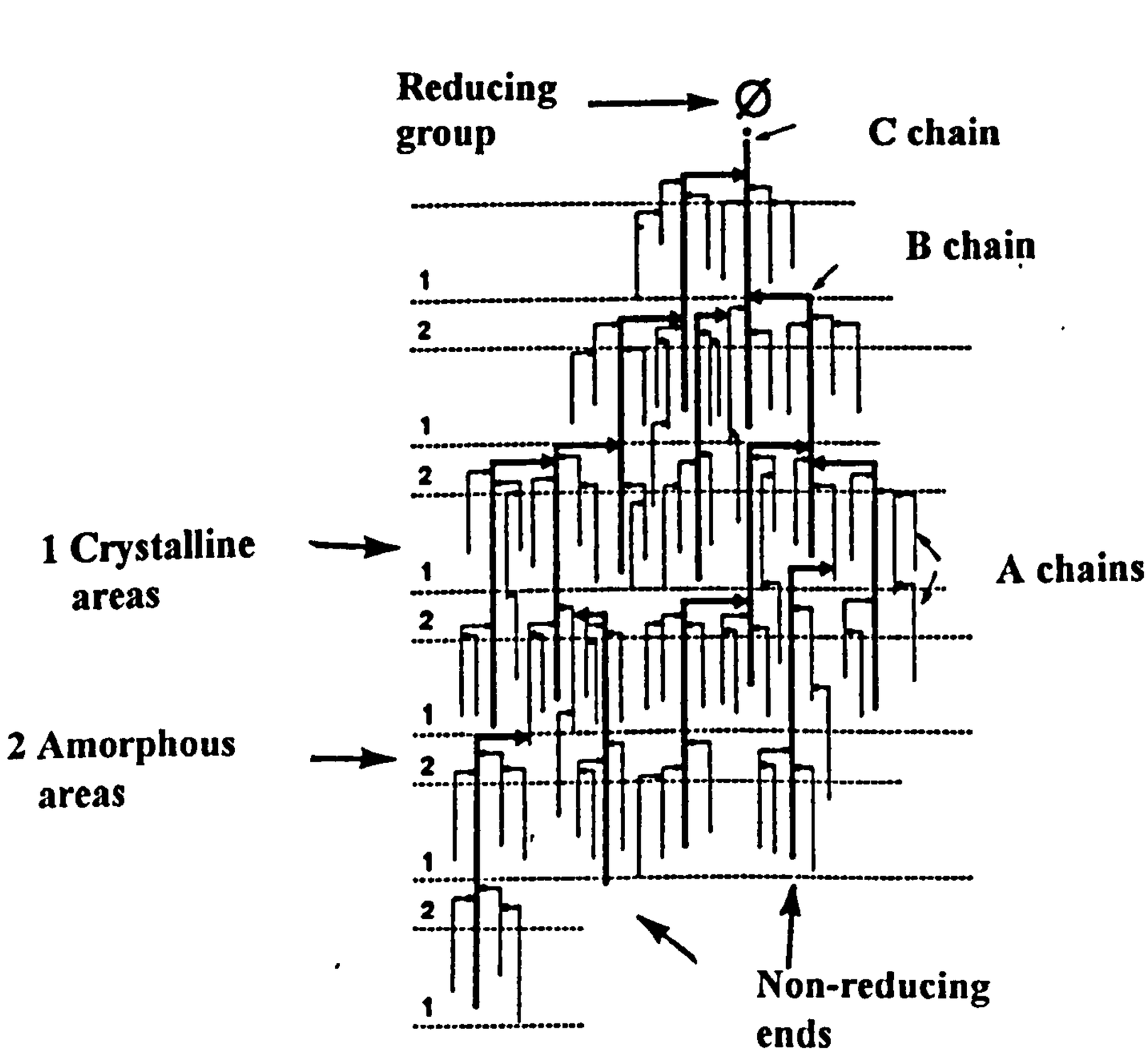


FIGURE 1. 5. AMYLOPECTIN 'CLUSTER' MODEL.



Adapted from Robin *et al.* (1974).

bush-like overall structure comprised by primary (A), secondary (B) and tertiary (C) α (1→4) chains (Figure 1. 5). The A chains are joined to the remainder of the molecule with a single α (1→6) bond, B chains are joined through a α (1→6) bond but may carry one more A and/or B chains on primary hydroxyl groups; the single C chain carries the sole reducing group (Ø). Amylopectin can exhibit some crystallinity but it is not as capable as amylose in forming organized crystals. These organized (crystalline) areas have been suggested to occur by a parallel association of several A chains to form 'clusters'. Each cluster presumably presents a molecular packing comparable to that existing with amylose crystals. Amorphous areas of the molecule are expected to arise at branching points where an ordered array is not possible.

(i). Starch granule organization

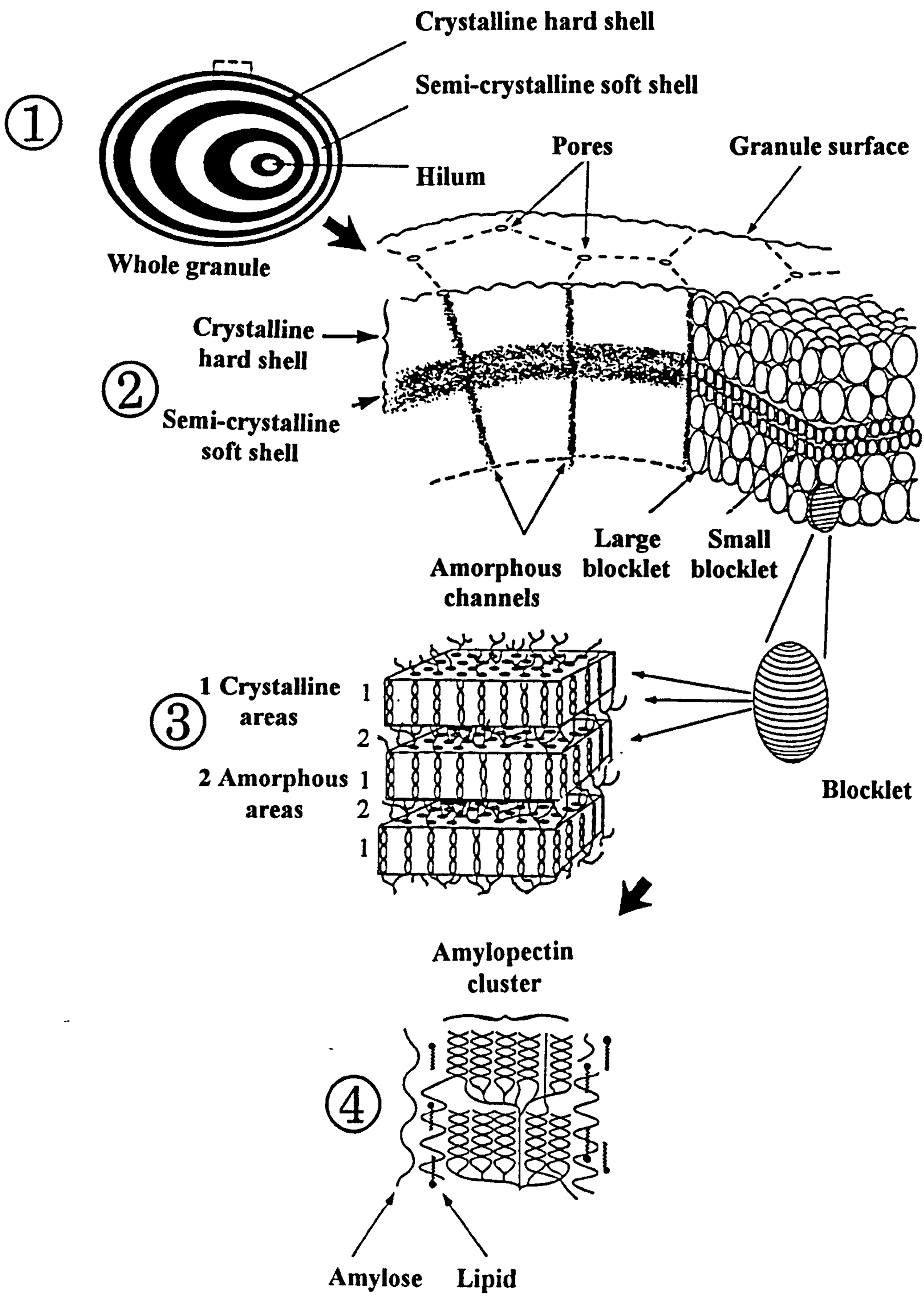
A mature cereal starch contains starch granules with different sizes. The synthesis of starch, growth and development of starch granules in the cereal endosperm were recently reviewed by Morell *et al.* (1995). Starch granules are synthesized by endospermic structures called amyloplasts. Bechtel *et al.* (1990) categorized wheat starch granules depending on their size into three types - A, B and C. Granules from type A have a diameter greater than 15.9 μm, granules from type B have a diameter between 5.3-15.9 μm and type C granules have a diameter less than 5.3 μm. At maturity, the total number of starch granules comprises 45.7% type C, 49.5% type B, and 4.8% type A granules. The type C granules constitute 3.4%, type

B 45%, and type A granules 51.6% of the total mass of starch in the wheat endosperm (Bechtel *et al.* 1990).

The shape of starch granules is dependent upon the proportions of amylose and amylopectin. Proportionally larger amounts of amylose, compared to amylopectin, in the starch leads to more rounded shapes whereas those containing more amylopectin are more flattened (Morell *et al.*, 1995). Most commonly, smaller granules tend to have higher levels of amylose than the big (type A) granules, and their shape is rounder (Bewley & Black, 1994).

The starch granule structure has been investigated and discussed over the last century, a review of the subject has been recently published by Gallant *et al.* (1997). They adduce arguments that there is a 'blocklet' organization within starch granules. The 'blocklets' are more or less spherical structures comprised of the amylopectin lamellas. The overview of starch granule structure is presented in Figure 1. 6 (Gallant *et al.*, 1997). At the lowest level of granule organization (Part 1), the alternating crystalline (hard) and semycristalline (soft) shells are shown (dark and light colours, respectively). The amylopectin is located both in the crystalline and semi-crystalline shells. In the semi-crystalline shells amylopectin is possibly in high interaction with amylose and its crystallinity is reduced. The shells are thinner towards the granule exterior (due to increasing surface area to be added to by constant growth rate) and the hilum is shown off centre. Part 2 shows that there are channels (channel system) in the granules. The channels are predominantly composed of semi-crystalline or amorphous material. Blocklet size is smaller in the semi-crystalline shells than in the crystalline shells. At the next highest level of structure (Part 3), one blocklet is shown containing several amorphous and crystalline lamellae. The next level (Part 4) shows

FIGURE 1. 6. OVERVIEW OF STARCH GRANULE STRUCTURE.



Adapted from Gallant *et al.* (1997).

amylopectin and amylose-lipid (and protein) organization in the granule structure.

The blocklets range in diameter from around 20 to 500 nm depending on starch type (botanical source) and location in the granule. Resistant starches such as a potato and high-amylose maize starch possess larger blocklets than less resistant starches.

(ii). Endosperm hardness

Wheat endosperm hardness is an important characteristic that plays a significant role in the marketing of wheat for bread making. Hardness of the endosperm affects the milling performance of wheat. Hard wheats shatter when milled and the flour is fine, with regular particle sizes and large surface areas. They yield angular endosperm particles in which starch granules are often damaged mechanically, and the plane of fracture of the endosperm frequently passes through the starch granules (Sulaiman *et al.*, 1993). Soft wheats tend to give flour with irregular particle sizes, lower surface areas and relatively little starch granule damage.

MacRitchie (1980) concluded that the major factors likely to be involved in endosperm hardness are the physical hardness of the starch and the protein, the strength of their interaction within the cell, and the interaction of individual cells to produce the overall grain structure. Two major theories on grain hardness have been discussed. First, endosperm hardness may be caused by the degree of starch-protein adhesion in the endosperm (Barlow *et al.*, 1973). Starch-protein adhesion could vary

in hard and soft wheat endosperm as a result of quantitative or qualitative differences in cellular products deposited at the starch-protein interface. It was shown that the softness may be caused by the presence of the 15-kDa protein called friabilin, which is isolated from the starch of soft wheats (Schofield & Greenwell, 1987). Hard wheats show a highly regular, strong matrix between starch and protein, which is the opposite to soft wheats (Sulaiman *et al.*, 1993). The second theory indicates that hardness depends upon the physical structure of the protein matrix (Stenvert & Kingswood, 1977). This theory holds that the degree of endosperm hardness is determined by the continuity of the protein matrix, its structure and the strength with which it physically entraps starch granules. It is probable that both starch-protein adhesion and protein matrix are involved in determining wheat hardness (Glenn & Saunders, 1990).

Recent research (Bechtel & Wilson, 1997) suggest that hardness develops as a result of endosperm senescence rather than accumulation of particular grain components. Senescence may cause changes in the starch granule surface such that surrounding components bind tightly in hard wheats, whereas the binding is weaker in soft wheats. Therefore, the surface of starch granules might be more important in determining hardness than the components with which the starch granules bind.

1. 2. 2. 2. Non-starch polysaccharides

The other major group of carbohydrates present in cereals are non-starch polysaccharides (NSP). NSPs have a structural function and account for approximately 10% of the whole grain. The majority of NSPs in cereals occur as

mixed-linked β-D-glucans and arabinoxylans (pentosans). Cereal β-D-glucans are linear polymers of glucose with β-(1→3), (1→4) glucosidic links (Figure 1. 7). Arabinoxylans are composed of two sugars, arabinose and xylose, in a more complex branched structure (Figure 1. 8).

The content and structure of polysaccharides vary both among cereals and significantly within different morphological parts of the grain (Theander *et al.*, 1993). Barley and rye contain more NSPs than other cereals (Table 1. 1). In comparison with barley, rye and oats, wheat contains less total and soluble β-D-glucans, and intermediate level of arabinoxylans. Maize is the cereal with the lowest level of arabinoxylans and β-D-glucans. There is evidence that there is substantial variation in the NSP content of different wheat samples (Table 1. 2).

Most NSPs are part of the cell wall and are closely associated with other polysaccharides or non-carbohydrate material such as protein and lignin (Fincher & Stone, 1986). The arabinoxylans of wheat are linked to lignin through a phenolic acid ester bond, and to proteins through a covalent link. The binding of the polysaccharides to other food components is important because it influences their solubility in aqueous media.

Recently the physicochemical properties of wheat non-starch polysaccharides have been recognized to cause some anti-nutritive activity. NSPs increase digesta viscosity and intestinal fermentation and decrease growth performance in young broilers (Choct *et al.*, 1996). The anti nutritive activity of wheat NSPs is not well understood, but adding xylanase significantly reduces the effects (Choct *et al.*, 1999). Younger chickens are more susceptible to the anti-nutritive effects of NSPs.

FIGURE 1. 7. MAJOR SOLUBLE NON-STARCH POLYSACCHARIDE OF BARLEY: β-(1→3), (1→4)-D-GLUCAN.

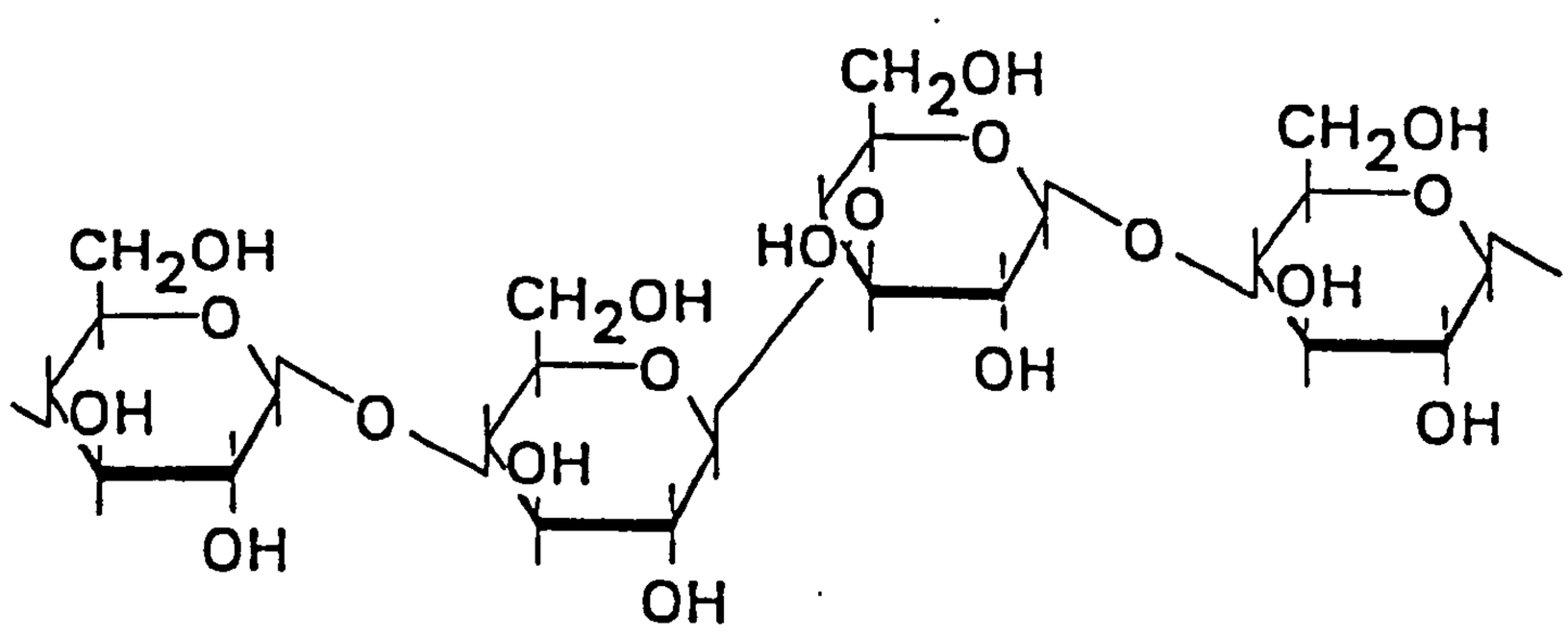


FIGURE 1. 8. MAJOR SOLUBLE NON-STARCH POLYSACCHARIDE OF RYE AND WHEAT: ARABINOXYLAN.

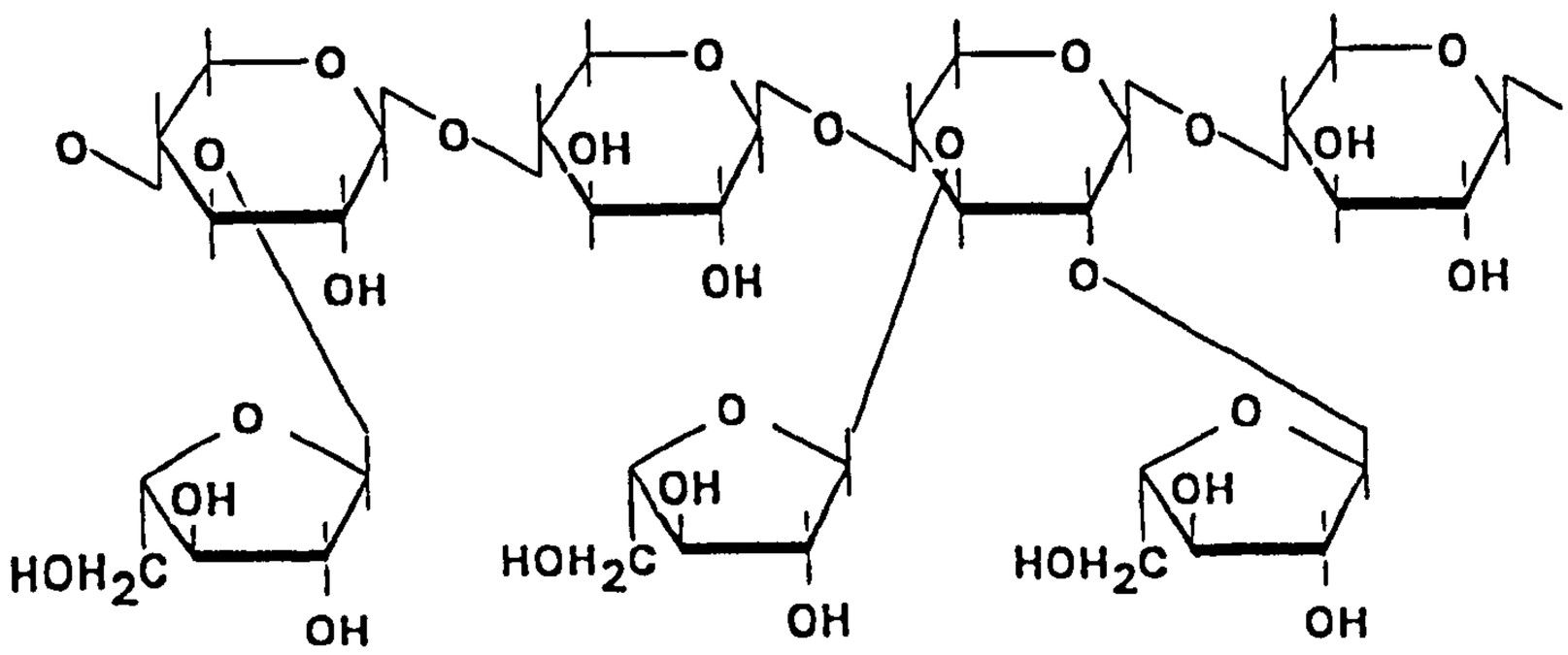


TABLE 1. 1. COMPARISON OF THE NON-STARCH POLYSACCHARIDE (NSP) CONTENT OF SOME CEREALS (g/kg DM).

NSP	Wheat	Maize	Barley	Rye	Oat
Soluble NSP	24 [1]	4 [1]	45 [1]	46 [1]	48 [1]
Insoluble NSP	90 [1]	16 [1]	122 [1]	86 [1]	30 [1]
Total NSP	114 [1]	20 [1]	167 [1]	132 [1]	78 [1]
Total arabinoxylans	60.5 [2]	42 [2]	33 [2]	89 [2]	76.5 [3]
Soluble arabinoxylans	10 [3]	-	7 [3]	25 [3]	5 [3]
Total β-D-glucans	5 [2]	1 [2]	76 [2]	12 [2]	33.7 [3]
Soluble β-D-glucans	5 [3]	-	29 [3]	7 [3]	21 [3]
Cellulose	20 [1]	5 [1]	39 [1]	15 [1]	7 [1]

From [1] Englyst *et al.* (1989); [2] Annison (1991); [3] Henry (1985).

TABLE 1. 2. COMPARISON OF THE RANGE OF NON-STARCH POLYSACCHARIDE (NSP) CONTENT OF SOME WHEAT CULTIVARS.

NSP	UK wheats [1] (g/kg as fed)	UK wheats [2] (g/kg as fed)	German wheats [3] (g/kg DM)	French wheats [4] (g/kg DM)	Australian wheats [5] (g/kg DM)
Soluble NSP	25-44	15-24	16-46	4-8	9-18
Insoluble NSP	97-111	66-107	35-90	51-72	72-142
Total NSP	132-142	83-129	75-115	55-78	81-157

From [1] Waldron (1997); [2] Austin *et al.* (1999); [3] Dusel *et al.* (1997); [4] Saulnier *et al.* (1995) (arabinoxylan content only); [5] Choct *et al.* (1999).

Choct *et al.* (1999) concluded that dry and hot weather during the grain filling phase of growth increases the NSP content of the grain and hence decreases the nutritive quality of wheat. Wet and cool weather had the opposite effect. Coles *et al.* (1997), also showed that drought conditions increased the level of β-glucans in wheat. This conflicts with the results of Dussel *et al.* (1997) who found that extract viscosity (presumably due to increased soluble NSP content) increased with increasing rainfall and lower temperatures during the growing period. The extract viscosity was positively correlated to the soluble pentosan content of the wheat. This environmental interaction with extract viscosity has not been investigated in detail. Extremes in the environmental conditions for growth of the wheat crop may be factors that affect the NSP content in wheat.

1. 2. 3. Protein

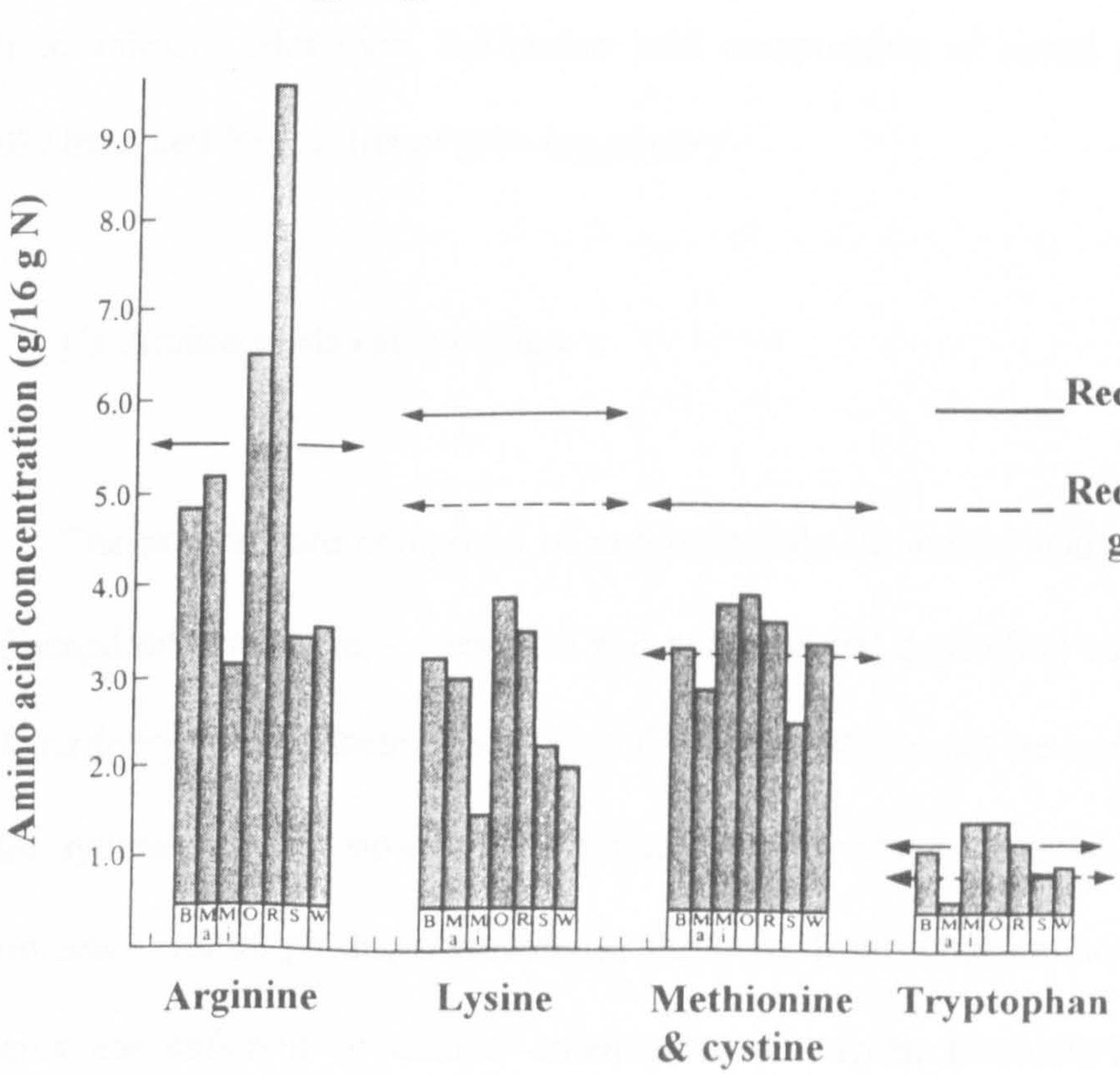
Proteins are complex organic compounds, and their content in harvested wheat ranges between 80-197 g/kg. According to Osborne's classification, seed proteins can be divided into four classes in relation to their solubility: albumins - soluble in water and neutral buffers; globulins - soluble in salt solutions; glutelins - soluble in dilute acid or alkali solutions; prolamins - soluble in aqueous alcohol. The approximate proportions of the main protein classes in some cereals are presented in Table 1. 3. The endosperm contains about 72% of the grain protein, aleurone layer 15%, pericarp and testa about 4%, scutellum 4.5% and embryo contains about 3.5% of the grain protein. The most important proteins present in wheat endosperm are prolamin and glutelin. The mixture of proteins present in the endosperm is often

TABLE 1. 3. THE APPROXIMATE AMOUNT OF PROTEIN (μg/g) IN SOME CEREALS AND THE COMMON NAME OF SOME STORAGE PROTEINS.

Protein	Albumin	Globulin	Prolamin	Glutelin
Cereals				
Wheat	90	50	400 (gliadin)	460 (glutenin)
Maize	40	20	550 (zein)	390
Barley	130	120	520 (hordein)	230 (hordenin)
Oats	110	560	90 (avenin)	230

From Bewley & Black (1994).

FIGURE 1. 9. MAIN LIMITING ESSENTIAL AMINO ACIDS OF CEREAL GRAIN PROTEINS (g/16 g N).



Adapted from McDonald *et al*. (1994).

named 'gluten'. All glutens possess the property of elasticity, and strong glutens are preferred for bread making. Conversely, strong gluten is not desirable in monogastric nutrition. Wheat, especially if finely milled, forms a pasty mass in the mouth and this may lead to digestive problems.

Over two-thirds of the albumin fractions of wheat is composed of multiple protein components capable of inhibiting α-amylases of diverse origin. Shainkin & Birk (1970) concluded that wheat amylase inhibitors do not seem to affect pepsin activity in the digestive tract. Although resistant to heat inactivation, wheat amylase inhibitors are readily destroyed by pepsin in the gizzard (Marchi *et al.*, 1977). Inhibitors in unprocessed wheat are more resistant to peptic action, but the extent to which they affect starch digestion *in vivo* is uncertain.

The concentration of protein in cereals is not sufficient to support the growth of farm animals. Moreover, the amino acid composition of cereal protein is not ideally balanced for the diet of growing poultry.

(i). Amino acids composition

The proteins are composed of approximately 22 amino acids. Amino acids are divided into two groups - essential and non-essential depending on the ability of chickens to synthezise them. The essential amino acids cannot be synthezised at all or are synthesized too slowly for the rate of requirement, whereas non-essential amino acids can be produced entirely in the body from other amino acids. Cereal proteins are deficient in certain essential amino acids, particularly lysine and methionine. A comparison of the main limiting amino acids between some cereals

and wheat cultivars is presented in Figure 1. 9, and Table 1. 4. The amino acid composition of cereals is influenced by the nature of the major storage protein. Albumins and globulins have a relatively good balance of essential amino acids, and as a consequence oats are a better source of dietary protein. In barley and maize grains, where prolamins are high, lysine is limiting, and tryptophan and threonine levels are also low. The major storage proteins in wheats are prolamins and glutelins which limits the lysine, methionine and arginine levels.

Quality of the storage protein is controlled largely by genotype and is achieved by cultivar selection. By contrast, grain protein percentage is influenced considerably by environment and management practice. Current research shows a big variation in protein content in commercially grown wheat cultivars: 9.5-22.8% (March & Biely, 1973), 9.7-15.7% (Davidson *et al.*, 1978), 10.2-17.3% (Coates *et al.*, 1977). The level of protein and the amino acids concentrations of wheat increased with the application of nitrogen (McNab, 1991). Lysine, methionine, threonine and cysteine contents were improved by the addition up to 350 kg nitrogen/ha.

(ii). Enzymes

Enzymes are proteins with high biological activity. The cereal grain contains a multitude of enzymes, the nature and proportion of which depends upon the stage of development of the crop. Starch degradation in germinating cereal seeds is the result of hydrolytic enzyme activity in the seeds. Complete hydrolysis of starch is

TABLE 1. 4. CRUDE PROTEIN (g/kg DM) AND AMINO ACID LEVELS (g/kg CP/DM) OF SOME WHEAT CULTIVARS.

Wheat cultivar	Aquila (UK) [1]	Copain (UK) [1]	Unknown (France) [2]	Inia 66 (Canada) [3]	Inia 66 (Canada) [4]	Pitic 62 (Canada) [3]	Pitic 62 (Canada) [4]	Glenlea (Canada) [3]	Glenlea (Canada) [4]
Crude protein	123	141	140	149	197	122	117	136	163
Amino acids									
Lysine	29.7	30.2	27.9	33.6	26.9	36.9	36.8	33.8	25.8
Methionine	20	20.8	16.4	30.8	21.8	31.1	25.6	33.1	23.9
Arginine	47.7	51.4	47.9	60.4	47.2	72.1	59.8	57.4	49.7
Leucine	64.4	69.2	73.6	70.5	64.5	79.5	67.5	72.1	64.4
Isoleucine	32.4	35.3	40.7	36.2	28.9	39.3	29.1	31.6	29.4
Histidine	22.4	24.4	22.1	24.8	24.4	27.9	27.4	22.8	23.3
Phenylalanine	46.2	50.1	48.6	48.3	42.1	54.9	36.8	49.3	42.9
Threonine	34.2	34.1	27.1	28.2	33	30.3	30.8	29.4	31.9
Valine	38.7	42.9	50	40.3	37.6	45.1	45.3	36.8	38

From [1] Fuller *et al.* (1989); [2] Green *et al.* (1987); [3] Salmon & Dunkelgod (1974); [4] Bell & Anderson (1984).

considered to result from the concerted action of four enzymes, α-amylase, β-amylase, debranching enzyme, and α-glucosidase (Sun & Henson, 1991). It is generally accepted that high α-amylase activity results in starch damage and is the most important enzyme associated with baking quality of wheats. The enzyme is only present in the mature grain in relatively small quantities and maintained at these level during storage under dry conditions. In damp stored wheat, the levels of α-amylase rapidly increases and leads to degradation of the starch. The baking industry indirectly measure the activity of α-amylase by measuring Hagberg falling number (Hagberg, 1961; Perten, 1964). Very low levels of α-amylase are required for baking wheat flour and a good quality sample has a Hagberg falling number (HFN) of >400s (Best & Muller, 1991). A low HFN (< 200s) is an indicator of pregermination in the grain. Degradation of starch by α-amylase results in deterioration of flour quality for the baking industry. It was found that feeding wheat with a high HFN gave better growth performances in broilers in comparison with wheat samples with low HFN (McNab, 1991; Rose *et al.*, 1993). HFN is not only dependant on α-amylase activity but also depends on other endosperm properties. Any other factor affecting the viscosity can give rise to a change in HFN.

(iii). Hormones

The growing seed contains other important protein substances called growth regulators, or hormones. The hormones auxin, gibberellins, cytokinins and abscisic acid (ABA) are found. In growing wheat they are probably involved in growth and development of the seed, the accumulation of the storage reserves, growth and

development of the extraseminal tissues, storage for later use during germination and early seedling growth and various other physiological functions.

1. 2. 4. Lipids

Lipids are minor constituents of wheat, but play major roles in wheat production, storage, processing and nutritional quality. The literature of wheat lipids was comprehensively reviewed by Morrison (1988), Morrison (1994) and Chung (1994) and most of the following information is from these papers. Wheat contains about 10-30 g/kg DM lipids, and it differs from other cereals, especially maize, in fatty acid composition (Table 1. 5). Cereal oils are unsaturated, they contain mainly linoleic and oleic acids, and because of this they tend to become rancid quickly. There is variation in the ether extract and fatty acid compositons between wheat cultivars (Table 1. 6). Lipids are unevenly distributed in wheat kernels. About 25-30% of wheat lipids are present in the germ, 22-33% in the aleurone layer, 4% in the pericarp, and 40-50% are in the starchy endosperm. This fraction has 20-31% nonstarch lipids and 16-22% starch lipids.

The wheat germ and aleurone fractions are relatively easily separated from milled flour as by-products due to their high-lipid contents. Lipids are partially transferred from the germ and aleurone in the form of tissue fragments and as oil adhering to the flour particle surface.

TABLE 1.5. FATTY ACID COMPOSITION OF SOME CEREALS.

Fatty acids (μg/g)	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Cereals	(16:0)	(18:0)	(18:1)	(18:2)	(18:3)
Maize [1]	120	20	240	610	< 10
Wheat [2]	170	< 10	110	630	70
Triticale [2]	170	< 10	130	610	80

From [1] Bewley & Black (1994); [2] Grela (1996).

TABLE 1. 6. ETHER EXTRACT AND FATTY ACID COMPOSITION OF SOME WHEAT CULTIVARS.

Fatty acids (g/kg)	Ether extract	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Wheat cultivar		(16:0)	(18:0)	(18:1)	(18:2)	(18:3)
Chinook	16.8	170	8	205	591	33
Inia 66	17.2	190	5	158	612	32
Glenlea	18.3	184	10	167	604	31
Pitic 62	18.6	190	8	142	622	35
Lemhi 53	20.5	206	9	151	596	34

From Salmon & Dunkelgod (1974).

1. 2. 5. Minerals and vitamins

All seeds contain minerals and vitamins. Wheat is a good source of α-tocopherols (vitamin E) and thiamin (B_1), but it is deficient in vitamin D, carotene (provitamin A) and riboflavin (B_2) (Table 1. 7).

Wheat is deficient in calcium, sodium, chlorine and many trace minerals compared to the requirements of poultry. Much of the phosphorus present in cereal grains is in the form of phytin which decreases its availability.

The minerals and vitamins are found in high concentrations in the aleurone layer (Table 1. 8). Whole wheat flour contains 3-10 times more iron (Fe), zinc (Zn) and copper (Cu) than white wheat flour (without bran and germ). Whole wheat contains more vitamins than wheat flour which is explained with the separation of the bran and germ in milling process.

1. 2. 6. Anti-nutritive constituents

Substances which can reduce the availability of nutrients can be classified as anti-nutrients. Wheat does not contain as many anti-nutrient factors as barley, rye or some legumes, but under some circumstances it is possible that wheat samples have some anti-nutritive effects. Trypsin inhibitors, lectins, alkyl resorcinols and tannins have been isolated from wheat , but at the concentrations found, they probably have no significant effect on poultry growth.

TABLE 1. 7. NUTRIENT SPECIFICATIONS OF BROILERS (6-8 weeks) FOR SOME MINERALS AND VITAMINS AND THEIR CONTENT IN WHEAT.

Minerals		Specifications	Content in wheat
Calcium	g/kg	8	0.5
Phosphorus, available	g/kg	3.5	1.1
Potassium	g/kg	3	4
Sodium	g/kg	1.5	0.4
Chlorine	g/kg	1.5	0.8
Magnesium	mg/kg	600	1000
Manganese	mg/kg	60	24
Zinc	mg/kg	40	28
Iron	mg/kg	80	40
Copper	mg/kg	8	7
Selenium	mg/kg	0.15	0.06
Vitamins			
Vit. E	IU/kg	10	13
Riboflavin	mg/kg	3.6	1.2
Pantothenic acid	mg/kg	10	11
Niacin	mg/kg	11	57
Vit. B_{12}	mg/kg	0.003	-
Choline	mg/kg	500	1002
Biotin	mg/kg	0.1	0.11
Folacin	mg/kg	0.25	0.4
Thiamin	mg/kg	1.8	4.3
Pyridoxine	mg/kg	2.5	4

From NRC (1984).

TABLE 1. 8. CONTENT OF SOME MINERALS (μg/g, DM) AND WATER SOLUBLE VITAMINS (μg/g) OF WHEAT AND MILLED FRACTIONS.

Fractions	Grain	Flour	Germ	Bran
Minerals [1]				
Zn	21-63	3.4-10.5	< 100-144	56-141
Fe	18-31	3.5-9.1	41-58	74-103
Mn	24-37	2.1-3.5	101-129	72-144
Cu	1.8-6.2	0.62-0.63	7.2-11.8	8.4-16.2
Se	0.04-0.71	0.01-0.45	0.01-0.77	0.1-0.75
Vitamins [2]				
Thiamin (B_1)	9.9	0.7	10.1	13.2
Riboflavin (B_2)	3.1	1.5	1.8	5.5
Niacin	48.3	9.5	23.5	171.4
Biotin	0.056	0.013	0.055	0.162
Folacin	0.56	0.09	0.59	1.59
Pantothenic acid	9.1	2.5	7	31.7
Pyridoxine ($B_{6)}$	4.7	0.48	5.3	13

From [1] Turnlund (1982); [2] Michela & Lorenz (1976).

1.3 AVAILABILITY OF NUTRIENTS OF WHEAT.

Small differences in the content or digestibility of nutrients in wheat can be economically important because of the high inclusion rates of wheat in many practical poultry feeds. The objectives of this section are to examine the availability to poultry of the major nutrients in wheat.

1.3.1. Carbohydrate availability

Starch

There have been numerous studies evaluating the digestibility in poultry of wheat starch (Table 1. 9). Studies with UK samples indicated that the starch is almost entirely digested by young and mature birds (Halnan, 1928; Bolton, 1955; Longstaff & McNab, 1986). Carre *et al.* (1995) and Steenfeldt *et al.* (1995) also found a high starch digestibility of between 0.9-1.0 in young and adult birds in wheat samples from Europe. Early work in Australia showed there were samples with poor starch digestibility (Mollah *et al.*, 1983; Rogel *et al.*, 1984). These data indicated that pelleting may improve starch digestibility in these situations.

Non-starch carbohydrates

The structural carbohydrates of the aleurone and endosperm cell walls comprise about 100 g/kg of the whole grain. Cell wall carbohydrates are a major

TABLE 1. 9. COMPARISON OF THE RANGE OF STARCH DIGESTIBILITY OF SOME WHEAT CULTIVARS.

Author		Country of origine	Starch digestibility	Age of birds	Starch procesing
Halnan (1928)		UK	0.86 - 0.90 [1]	-	unpelleted
Bolton (1955)		UK	1.0	2 weeks	unpelleted
Longstaff & McNab (1986)		UK	0.995 - 0.999	mature (cockerels)	unpelleted
Steenfeldt *et al.* (1995)		Denmark	0.985 - 0.993	mature (cockerels)	unpelleted
Mollah *et al.* (1983)		Australia	0.80 - 0.99	mature (cockerels)	unpelleted
Rogel *et al.* (1984)	# 1	Australia	0.818 - 0.999	6 weeks	pelleted
	# 2	-	0.569 - 0.961	3 weeks	unpelleted
	# 2	-	0.947 - 0.994	3 weeks	pelleted
Annison (1990)		Australia	0.931 - 0.982	5 weeks	unpelleted

[1] As nitrogen free extract.

source of energy for ruminants, but there is conflicting evidence of the extent of their digestion by poultry. Halnan (1928), reported variation in cell wall carbohydrate digestibility between 0.10-0.12. Bolton (1955) obtained about 0.33 digestibility for wheat pentosans, and 0.0 for cellulose and lignin. Longstaff & McNab (1986) recorded variation between 0.18-0.30 in wheat pentosan digestibility, and about 0.66 and 0.36 digestibility for hemicellulose and cellulose, respectively. Carre *et al.* (1990) reported that the digestible fraction of the NSP corresponds to the soluble part, whereas the insoluble NSP was not digested by adult cockerels. The same authors found that wheat NSP digestibility ranged between 0.13-0.22 for cockerels, and between 0.08-0.18 for ducks. Rats were able to digest NSP from 0.44 to 0.86. Steenfeldt *et al.* (1995) reported that apparent digestibility of wheat NSP varied between 0.11-0.57 in adult cockerels. Diets containing more soluble NSP had higher digestibility.

Recent research suggests that the digestibility of NSPs could depend on the ages of the birds (Carre *et al.*, 1995). NSPs had a higher digestibility in mature cockerels than in young chickens (Carre et al., 1995). McNab (1973) also found that the ability of the birds to digest pentosans increased with their age. In an experiment with intact and caecectomized cockerels, Carre & Gomez (1994) observed that NSP digestibility was greater in intact birds. Soluble NSPs were digested both in the caeca and in more proximal parts of the small intestine. Choct *et al.* (1996) demonstrated that increasing cell wall polysaccharides increased ileal fermentation. Carre & Gomez (1994) also showed that the major part of organic acids produced from fermentation were absorbed in the caeca. In this case the increasing importance of the caeca could be a cause of increased NSP digestibility in mature birds.

1.3.2. Protein and amino acids availability

Lysine and threonine have a lower apparent digestibility than the other amino acids contained in wheat protein (Table 1. 10), although the reduction in true amino acid digestibility coefficients of these two amino acids is less marked. However, McNab (1991) obtained low coefficients for the true digestibility of lysine and threonine, 0.81 and 0.82 respectively, whereas the digestibility of the other amino acids varied between 0.88 - 0.91. The low availability of lysine and threonine is similar to other cereals; Ravindran *et al.* (1999) found low lysine and threonine digestibility in wheat, maize and sorghum, and no significant differences between the three cereals.

Fuller *et al.* (1989), compared two types of wheat, and concluded that the wheat cultivar affected amino acid digestibility. The amino acids in high-protein wheat cultivars was, on average, 6% more digestible than that in the low-protein cultivar. Similarly, the digestibility of the wheat protein was lower in the low-protein cultivar. However, Sauer *et al.*, (1981) found no significant differences in the essential amino acid digestibilities for growing pigs between different wheat samples. Increasing the nitrogen fertilizer application to the growing wheat crop decreased the crude protein digestibility in high protein wheats, but it increased protein digestibility in low-protein cultivars (Fuller *et al.*, 1989).

Ernest *et al.*, (1992) reported that inactivation of α-amylase inhibitors in wheat by steam treatment improved protein digestibility in broilers by 6.5%.

TABLE 1. 10. APPARENT (AD) AND TRUE DIGESTIBILITY (TD) OF PROTEIN AND AMINO ACIDS IN SOME WHEAT CULTIVARS IN POULTRY AND PIGS.

Wheat cultivar	Glenlea (Canada) [1]		Unknown (France) [2]		Neepawa (Canada) [3]		Aquila (UK) [4]		Copain (UK) [4]	
	AD	TD	AD	TD	AD	TD	AD	TD	AD	TD
Crude protein	-	-	0.75	0.89	0.86		0.63	-	0.71	-
Amino acids										
Lysine	0.71	0.91	0.50	0.80	0.77	0.84	0.52	0.62	0.63	0.71
Methionine	0.82	0.93	0.78	0.90	0.82	0.85	0.75	0.80	0.83	0.87
Arginine	0.82	0.93	0.70	0.86	0.87	0.94	0.63	0.73	0.72	0.80
Leucine	0.85	0.94	0.80	0.91	0.89	0.93	0.68	0.73	0.76	0.80
Isoleucine	0.85	0.94	0.78	0.92	0.87	0.92	0.65	0.71	0.74	0.79
Histidine	0.82	0.95	0.64	0.82	0.86	0.91	0.58	0.63	0.73	0.76
Phenylalanine	0.90	0.96	0.81	0.91	0.88	0.94	0.71	0.76	0.78	0.82
Threonine	0.73	0.91	0.47	0.85	0.77	0.86	0.45	0.55	0.66	0.73
Valine	0.83	0.93	0.74	0.90	0.80	0.85	0.61	0.65	0.72	0.75

From [1] Sibbald (1978) (with poultry); [2] Green *et al.* (1987) (with poultry); [3] Sauer *et al.* (1981) (with pigs); [4] Fuller *et al.* (1989) (with pigs).

1.3.3. Lipids, vitamins and minerals availability

Wheat contains about 10-30 g/kg (DM) lipids. Lipids supply only approximately 3-7 % of the available energy of wheat. There is no published information on digestibility of these lipids although it is probable that they are highly digestible. Changes in the lipids of wheats stored under suitable conditions are very slow and their feeding quality changes very little during storage. In wheat stored in high humidity conditions, lipolysis is faster and it leads to loss of feeding and baking quality. Ernest *et al.* (1992) reported that steam treatment improved fat digestibility by about 4% in wheat-fed broilers.

Availability of fat soluble vitamins in wheat is connected with the lipid availability. Some deterioration of the lipids in wheat will decrease the availability of the fat soluble vitamins (Sokola, 1991). Inappropriate storage conditions may decrease the concentration and bioavailability of the vitamins in cereal grains.

Although many of the water soluble vitamins are present in the bran, these vitamins appear to be highly available. Ristow *et al.* (1982) found that bran had no detectable effect on the utilization of vitamin B in chickens and similar results were found by Keagy and Oace (1984) using rats.

Much of the phosphorus present in wheat grain is in the form of phytates. Phytin is the insoluble mixed phosphorus, potassium, magnesium, and calcium salt of myo-inositol hexaphosphoric acid (phytic acid). Phytic acid and its conjugates are generally regarded as being nutritionally undesirable since they can bind essential dietary minerals (e.g., Zn, Ca, Fe, Cu, Mn), thus making them wholly or partially

unavailable for absorption (Davies & Nightingale, 1975). The phosphorus from calcium phytate is utilized only 10% as effectively in young chickens as the phosphorus from disodium phosphate. In laying hens, phytate phosphorus was only 50 % as available as dicalcium phosphate.

Supplementation of dietary phytase increased the availability of phytate phosphorus and calcium and improved growth performances in poultry (Qian *et al.*, 1997). Supplemental vitamin D_3 also increased the digestibility of phytate phosphorus and total phosphorus and the calcium retention in the chick. Phytase supplementation may increase bioavailability of the trace minerals (Qian *et al.*, 1996 a; 1996 b).

Turnlund (1982) concluded that, in general, the minerals are less available from whole grain products than from more refined products. Van Dokkum *et al.* (1982) concluded that increasing the amount of bran in bread did not affect the mineral balance, but decreased the availability of minerals in human subjects.

1.4. MEASUREMENT OF ENERGY AVAILABILITY IN POULTRY FEEDS INCLUDING A QUANTITATIVE REVIEW OF SYSTEMS OF NET ENERGY EVALUATION.

The concentrations of utilisable energy per unit of price is important information to allow choices to be made between feedstuffs for practical poultry feed formulation. There has been a long lasting debate as to whether Net Energy (NE) or Metabolisable Energy (ME) is preferable.

1. 4. 1. Metabolisable Energy

Metabolisable energy (ME) is a common measure of energy availability of poultry feeds. Different methods of ME determination have been comprehensively reviewed by Farrell (1979), Farrell (1981), Sibbald (1982), Cooke (1987), Fisher and McNab (1987) and McNab and Blair (1988). The presented information that is not specifically referenced in the text has been derived from the sources above.

Three general types of ME balance experiments are performed:

(i) Assays that involve preliminary feeding periods to establish 'equilibrium' conditions. Complete diets must be used in most cases and substitution methods used for ingredients.

(ii) Rapid assays, using feed withdrawal before and after giving a known portion of test feed. The birds are given a free access to the feed for a short time only.

(iii) Rapid assays, as above, but using tube-feeding to place the test feed directly into the birds crop.

The first type of assays (type i) are most frequently used to measure Apparent Metabolisable Energy (AME) in the poultry feed. AME values are calculated from data generated from experiments using young broilers, kept in metabolism cages and fed ad libitum. Usually a 8-14 day feeding period is used with a preliminary feeding period in which birds adapt to the test feed. In the last 3-5 days, total feed intake is measured and all excreta are collected. The AME of the diet is calculated by determining the total amount of energy contained in the feed consumed by the bird, and the amount of energy excreted, and calculating the difference that is retained by the bird for growth and maintenance (Hill & Anderson, 1958; McDonald *et al.*, 1994).

Equation 1.1. AME calculation:

$$\text{AME (MJ/kg)} = \frac{(E_{\text{FEED INTAKE}}\text{ (MJ)} - E_{\text{EXCRETA}}\text{ (MJ)})}{\text{Feed intake (kg)}}$$

The AME of feed will vary according to whether the amino acids it supplies are retained by the birds for protein synthesis or are deaminated and their nitrogen

excreted. For this reason, AME values are sometimes corrected to zero nitrogen balance, by deducting 34.39 kJ for each 1 gram of nitrogen retained (Hill & Anderson, 1958).

Equation 1.2. Nitrogen-corrected AME calculation:

$$AMEn\ (MJ/kg) = \frac{(E_{FEED\ INTAKE}\ (MJ) - E_{EXCRETA}\ (MJ)) - (N_{RETAINED}\ (kg) * 34.39\ MJ/kg)}{\text{Feed intake (kg)}}$$

Farrell (1978) proposed a rapid assay (type ii) for AME determination using mature cockerels. The birds were trained to consume satisfactory intakes in 1 hour after 23 hours starvation. When feeders were removed from the trained birds after 1 hour, a plastic sheet was placed on each tray and excreta were collected for the next 24 hours. A major advantage of this method is that a result can be obtained within 36 hours of receipt of a sample (Farrell, 1978).

AME values vary with respect to feed intake. Sibbald and other scientists developed a rapid assay (type iii) for a True Metabolisable Energy (TME) determination using mature cockerels and a modified procedure has since been proposed by McNab and Blair (1988). They recommended a 48 h starvation of the experimental birds to ensure that their gastrointestinal tracts are as empty as possible at the start of the assay. In order to partly alleviate the effect of starvation, all birds are given two doses of 25 g glucose about 40 and 16 h before tube-feeding. It was shown that feeding about 50 g glucose to starved birds significantly decreased the exogenous energy losses and nitrogen losses and improved the repeatability of the results. Birds from which endogenous energy losses (EEL) are to be derived, are fed

50 g glucose rather than given no food. The other birds are tube-fed 50 g of a test feed. The cockerels may have a low or variable water intake during the assay, so all birds are given 50 ml water by tube about 32 h after feeding. After the test feed is given, excreta are collected for the next 48 hours. EEL can be corrected to zero nitrogen retention. (Equation 1.3).

Equation 1.3. Nitrogen-corrected TME calculation:

$$\text{TMEn (MJ/kg)} = \frac{(E_{\text{FEED INTAKE}}\ (\text{MJ}) - E_{\text{EXCRETA}}\ (\text{MJ})) + \text{EEL (MJ)} - (N_{\text{RETAINED}}\ (\text{kg}) * 34.39\ \text{MJ/kg})}{\text{Feed intake (kg)}}$$

There is a close relationship between AMEn and TME values. Comparing the TME and AMEn values of 13 different feedstuffs, Sibbald (1977) found that TME:AMEn ratio is 1.097. Similarly, Halloran (1980) found that the TME:AMEn ratio varied between 1.12-1.16. Jonsson and McNab (1983) observed that TME:AMEn ratio was 1.166 feeding poultry with diets containing grass meal. Farrel (1978) did not find any differences in ME when comparing continuously (type i) and rapid (type ii) methods of feeding.

Sibbald (1978) examined the effect of age of bird on the TME of different diets and concluded that TME values obtained with adult roosters can be used in the formulation of diets for young broilers. There was a generally good agreement in the TME values between roosters, broilers, and poults although the values for broilers tended to be slightly lower than for roosters and poults (Dale & Fuller, 1980). Shires *et al.* (1980) found no difference due to age of bird in TME of several feedstuffs except for rapeseed meal.

1. 4. 2. Net Energy

Net energy is the metabolizable energy of the food corrected for the energy losses that result from the assimilation of the dietary nutrients. This energy loss is frequently termed the heat increment of digestion. The remaining NE is available for maintenance and production. Growth and egg production are the only products of NE that do not result entirely in heat emission.

During the early years of poultry nutrition research, there was a debate on the preferred system for describing the available energy content of feedstuffs. Digestible energy determinations were not favoured because of the practical problems of separating the intestinal and urinary components of the excreta, so the main focus of the debate was on the relative merits of net energy (NE) and metabolizable energy (ME) systems.

Fraps (Fraps & Carlyle, 1939; Fraps 1946) produced the first comprehensive data set of energy availability in feedstuffs by determining their productive energy concentrations. Productive energy (hereafter termed NEp) is a form of net energy. A comparative slaughter technique was used to measure energy deposition in growing chickens (Fraps & Carlyle, 1939). A number of techniques were used to avoid some limitations of the methods of determining NE: First, the net energy of a feed depends on the composition (total amount of protein and fat) in the carcass growth or egg output. Fraps used only young, growing chickens that were depositing high proportions of lean meat compared to carcass fat and the lean:fat ratio remained similar between experiments. Second, it is difficult to introduce the test feedstuff

into a diet formulation without affecting the overall nutrient balance of a feed. Fraps substituted cereals and cereal by-products for maize and substituted high protein feeds for a mixture of maize and casein. Third, a correction must be made for possible differences in the maintenance component of the total heat output of the birds used in an experiment to determine NE. A high maintenance requirement would reduce the amount of energy that would be available to the bird for productive output. The NEp technique provided the experimental feeds at two daily intake levels to different groups of chickens within each experiment. Maintenance requirement was predicted by using simultaneous equations assuming that the requirement was directly proportional to the body weight of the birds.

The NEp of an individual feedstuff thus indicates the efficiency of energy utilization for growth of young chickens depositing mostly lean meat. The data therefore has relevance to the requirements of the modern broiler chicken industry. The NEp data produced in these experiments were used by some nutritionists within the USA as a practical method of formulating poultry feeds up to the early 1970s (Jensen, 1977) although ME systems were predominantly used from the early 1960s onwards (de Groote, 1974b).

Even before Fraps (1946) published all of his data, research had indicated that metabolizable energy would be a preferred system of evaluating the available energy concentration of poultry feeds. Axelsson (1939) examined other NE systems (Starch units and Scandinavian Feed Units) and concluded that metabolizable energy provided a more suitable basis for feed evaluation because it was relatively independent of the nutritional quality of the test diet. Halnan (1951) also demonstrated that the determined NEp of a cereal depended upon the protein quality

of a test diet and concluded that a ME system was more suitable for poultry feed evaluations. Davidson *et al.*, (1957) and Hill and Anderson (1956) examined the NEp protocol and found high variability between individual birds within dietary treatments. Hill and Anderson (1956) directly compared NEp and ME determinations and observed that the variability of the ME determination was much lower although de Groote (1974b) considered that some NE techniques had satisfactorily low variability. The NEp values produced by Fraps indicated that there was a low efficiency of utilization of ME for production compared to other species. This could reflect an overestimate of the energy used for maintenance by the chickens and the size of any overestimate would have been increased by the relatively slow growth rates of the birds used in these NEp determinations (Fraps & Carlyle, 1939) compared to modern poultry meat strains. Whilst the poor growth was probably due mostly to genetic differences between the birds and modern chicken strains, an inappropriate nutrient specification in the diets may have also contributed. Titus (1955) demonstrated that many of the digestibility coefficients determined by Fraps were not consistent with other estimates of nutrient digestibility. However, Fraps' work was begun in the 1920s and many changes would have occurred in the processing methods and overall chemical composition of many of these feedstuffs compared to the post-war years.

In summary, Fraps' data have received a great deal of scrutiny since their publication in 1946. Although the values obtained for the NEp of feedstuffs were low relative to predicted values, his methodology still gives a reliable comparison of NEp in a large range of feedstuffs with additional information on nutrient digestibility. The high variability between individuals in comparative slaughter

experiments makes this type of NEp determination laborious and expensive. Fraps' data remain, however, as the only comprehensive published information on NEp and nutrient digestibility of a large range of poultry feed ingredients.

The ME system of describing the energy concentration of poultry feeds is now widely used. However, the ME system has a number of limitations in comparing the economic value of feeds ingredients as sources of utilizable energy for poultry. Variations in the proportion of fat, starch and protein affect the efficiency of utilization of the ME. The efficiency of utilization of ME derived from fat is greater than that from carbohydrates and the efficiency of utilization of ME from protein is lower than that of carbohydrates (Carew & Hill, 1964; Hoffmann & Schiemann, 1971; De Groote *et al.*, 1971). De Groote (1975) showed that a system based on ME values underestimates the utilizable energy of fats and fat-rich feedstuffs and overestimated protein-rich feedstuffs in comparison with carbohydrates.

Modern poultry meat production is now highly competitive and small differences in the efficiency of utilization of the supplied feed can be economically significant. Although ME systems have given estimates of energy availability that have acceptably low variability and high repeatability, it is possible that further progress in describing and understanding the differences in energy availability between feeds may only be achieved by using a net energy system.

No large datasets of determined net energy values have been published since those of Fraps (1946). Determined ME values and nutrient digestibility coefficients are now the only empirical data that are generally available to poultry nutritionists with which to evaluate the energy availability of feedstuffs. Recent approaches to

determine the NE of poultry feeds have therefore used methods where NE is predicted from the determined nutrient availability or ME of the individual feeds.

The efficiency of utilization of energy in broiler chicken feeds is affected by a variety of factors. Systems of modelling the net energy contribution of different feeds have been proposed (MacLeod, 1994). However, modern poultry production methods have resulted in a less complex problem. There is now a remarkable similarity of intensive broiler production systems around the world. A small number of multinational poultry breeding companies supply a high proportion of all commercial broiler stocks. The majority of broiler chickens supplied by the different companies have similar growth potential and body compositions. Most intensive broiler production units have some amount of temperature control so that well-defined, economically-efficient temperatures are maintained. Equipment and husbandry methods also tend to be uniform such that growth, feed utilization, and body composition of broiler chickens are similar from production sites around the world. The proportion of utilizable energy that is used for maintenance and the proportion of lean and fat in the body weight gain could therefore be expected to be approximately constant in most systems of intensive broiler production. The main variable that a net energy system needs to describe is the efficiency of utilization of feed ingredients and complete feeds.

Nehring *et al.* (1969) proposed that the NE of a poultry feed or individual feedstuff could be predicted from the composition of digestible starch, protein and fat. They used nutrient balance and calorimeter studies to quantify the different efficiencies of utilization of the three nutrients. The studies used adult birds that were predominantly depositing fat in the body weight gain and so they termed this

value Net Energy for fat deposition (hereafter called NEf). However, they observed that the prediction equation was improved if corrections were made for the concentrations of milk protein, milk fat, monosaccharides and disaccharides. The correction for milk protein (+4.2MJ/kg) could be explained by previous observations that amino acid balance has large effects on determined NE (Halnan, 1951). Reductions in the predicted NEf were proposed for dietary concentrations of digestible monosaccharide, disaccharide and milk fat and these corrections do not appear to have any biological logic.

De Groote (1974a) reviewed the literature that described the efficiency of utilization of nutrients by growing poultry. He proposed that the efficiency of utilization of digestible carbohydrates, fats, and protein were 0.7, 0.9 and 0.6 respectively. A net energy concentration of a poultry feed (hereafter termed NEue) can be derived by multiplication of these efficiency coefficients with the gross energy of the individual digestible nutrients. Modern poultry feeds are unlikely to include any significant amounts of milk products or simple sugars, so the approaches of Hoffmann and Schiemann (1980) and de Groote (1974a) were similar. De Groote (1974a) proposed a much higher efficiency of utilization for digestible crude protein. However, the birds used by Hoffmann and Schiemann (1980) were depositing predominantly fat and it is possible that the efficiency of utilization of digestible crude protein is less efficient in this type of chicken compared to growing chickens that are depositing a high proportion of carcass protein.

Emmans (1994) proposed a theoretical model that resulted in a correction to determined ME values that he termed effective energy (hereafter termed NEee[1]). First, he proposed that ME derived from protein had a significantly lower efficiency

of utilization and that an increasing proportion of digestible crude protein reduced the NEee[1]. Second, he proposed that high faecal energy losses were associated with high heat increments of digestion. A reduction of the determined ME for the amount of excreted faecal organic matter therefore gave an improved estimate of the effective energy. Third, he proposed that, if dietary fat were used directly for lipid growth, there was a reduction of heat increment of 12MJ/kg. Emmans (1994) examined other poultry experiments and proposed that 0.3 of body fat came directly from feed lipid. An increase of 4MJ/kg (12 x 0.3) for the effect of direct transfer of dietary lipid was proposed.

Emmans (1994) also proposed another correction of determined AME values (hereafter termed NEee[2]) that he derived empirically from statistical analysis of hen data: the correction involves a reduction of the AME of a feedstuff for increasing amounts of crude protein. Modern broiler chicken feeds have high energy densities so the amount of faecal organic matter is relatively low. Although added dietary fat is frequently used in broiler feeds, the amounts are relatively low otherwise problems would result with soft and crumbly pellets and the poor flowing of feed through automatic feed delivery equipment. Variation in the crude protein content of broiler diets is therefore the major variable that would affect the relationship between AME and the effective energy concentration. The two equations proposed by Emmans (1994) therefore have a logical similarity.

No quantitative evaluation has been published of the accuracy of these predicted net energy values in comparison to using ME. De Groote (1974a) showed that diets formulated using predicted net energy values were economically more efficient compared to diets formulated using ME values. Sondakh *et al.*, (1978) used

similar methods to formulate two series of poultry feeds using predicted NE or ME data, but they found no advantage to using a NE system. However, these types of comparison just examined the economic advantages of using different dietary energy and protein concentrations under different feedstuff price structures and did not demonstrate the predictive accuracy of the Net Energy system. There is still a need to test whether there is a good relationship between determined AME and predicted NE concentrations of individual feedstuffs for poultry. The NEp data published by Fraps included determined nutrient compositions and digestibility coefficients for fat, protein and nitrogen-free extract so the AME of each feedstuff could be calculated. This method for calculating AME gives an increased experimental variability but still provides a reliable estimate of the AME of a feedstuff. Comparisons of the relationships between ME, predicted NE and determined NE values are possible by using these data.

The objectives of this section are to statistically evaluate the relationship between ME and determined NEp concentrations of the range of feedstuffs examined by Fraps (1946). Second, to statistically compare four proposed equations that predict the NE of feedstuffs with the determined NEp values of Fraps (1946).

1. 4. 2. 1. Methods of data review

Comparison of AME with determined NEp

Fraps (1946) published data for NEp and digestion coefficients of crude protein, ether extract and nitrogen-free extract for 62 individual feedstuffs. In the present study, the AMEs of the 62 feedstuffs were calculated by multiplying the concentrations of digestible crude protein, ether extract and nitrogen-free extract contents by their gross energy concentrations (22.4, 39.2 and 17.2 MJ/kg respectively). The sum of the three gross energy contributions was considered the AME concentration of the feedstuff. Implicit in these calculations was the approximation that crude fibre had no AME content.

The relationship between AME and NEp was examined using linear regression with AME as the explanatory variable and NEp as the response variable. There was no evidence of curvature in any of the regression analyses in this study. Unaccountable variation in all regression analyses in this study was estimated by calculating the standard error of observations ((residual mean squares)$^{0.5}$) and the percent variance accounted for (adjusted R^2 statistic) that was calculated as (100 x (1 – (residual mean squares)/(total mean squares)) from the analysis of regression (Genstat 5 Committee 1987).

The 62 feedstuffs used by Fraps covered a wide range of AME values but a high proportion had low AME concentrations. These low AME feedstuffs are unlikely to be now used in commercial poultry feed formulations because of modern methods of feeding high nutrient density feeds to growing broiler chickens. In addition, a subset of feedstuffs was examined with a range of AMEs that was more typical of currently used feedstuffs. The range of AME concentrations selected was 8-18 MJ/kg and 45 of the feedstuffs were within this range. Five of these were dried vegetable products that are also unlikely to be used in modern proprietary poultry

feeds, so these were also eliminated from the data set. The remaining 40 feedstuffs were classified into four groups; cereals and high starch foods, vegetable high protein feeds, animal high protein feeds and cereal by-products (Table 1. 11). A second linear regression analysis was performed on these data and the hypothesis was tested that the relationship of AME or predicted NE with determined NEp differs according to the feedstuff category.

Calculation of NE from prediction equations

Four prediction equations were evaluated (Table 1. 12). The AME concentrations for the feedstuffs, calculated as described in the previous section, were used in the calculation. The nutrient digestibilities and crude protein concentrations of the feedstuffs, as described by Fraps (1946), were also used.

The concentrations of digestible protein, ether extract and nitrogen-free extract (assumed to be available carbohydrate) were used to derive the NE value described by De Groote (1974a). A similar method was used for the NEf values but this calculation required data for digestible crude fibre that were not provided by Fraps (1946). However, Titus (1955) published crude fibre digestibility coefficients for a similar range of feedstuffs and, although the nutrient compositions of the feedstuffs may have been different (as discussed previously), these values were used as the best available estimate. Some feedstuffs were not evaluated by Titus (1955), so assumed values for crude fibre digestibility were used (obtained by Fraps, 1946 for similar feedstuffs). The assumed percentage digestibility coefficients were as

TABLE 1. 11. CLASSIFICATION OF 40 FEEDSTUFFS AND THEIR DETERMINED AME AND NEp VALUES IN MJ/kg (in parentheses, respectively).

Cereals and high starch foods	Vegetable high protein foods	Cereal by-products
Barley (11.9;7.5);	Beans, all kinds, cooked (10.9;7.3);	Wheat gray shorts (10.4;6.6);
Barley, no hulls (12.5;8.7);	Coconut oil meal (8.5;5.7);	Maize gluten feed (269 CP) (8.0;5.2);
Broom corn seed (10.6;7.5);	Cottonseed meal (10.9;6.4);	Maize gluten meal (446 CP) (12.5;7.7);
Buckwheat flour (14.1;8.6);	Linseed oil meal (8.2;5.1);	Rice bran (11.3;7.1);
Cane seed (13.7;8.7);	Soybean oil meal (10.7;5.9);	Rice polishings (14.7;9.5);
Sorghum (14.6;10.2);	Soybean oil meal, low fat (9.5;5.2);	
Hegari grain (14.1;9.6);	Sunflower (12.8;8.7);	
Maize (14.9;10.5);		
Feterita, grain (14.2;9.9);	<u>Animal high protein feeds</u>	
Millet seed (12.9;9.1);		
Milo grain (15.0;10.5);	Blood meal (16.5;9.2);	
Oats (10.0;7.0);	Buttermilk, dried (12.4;6.5);	
Oat groats (15.5;10.7);	Yeast, dried (10.7;4.4);	
Potatoes, white dried (12.5;8.2);	Meat and bone meal (11.0;6.7);	
	Meat meal (610 CP[1]) (12.7;7.8);	
Potatoes, sweet dried (13.1;8.5);	Meat meal (650 CP) (11.2;6.6);	
	Fish meal (13.4;8.5);	
Rice (11.2;7.1);	Milk, dried skim (11.8;4.8);	
Rice polished (14.8;9.6);	Tankage (530 CP) (11.2;6.5);	
Rye seed (11.5;7.5);		
Wheat flour (13.7;8.8);		

[1] CP - crude protein concentration (g/kg).

TABLE 1. 12. EQUATIONS USED TO ESTIMATE THE NET ENERGY OF POULTRY FEEDS.

Authors	Equations to calculate net energy
Hoffmann & Schiemann (1980)	NEf (MJ/kg) = 10.80DCP + 33.5DEE + 13.4(DNFE+DCF) [1]
De Groote (1974)	NEue (MJ/kg) = 13.4DCP + 35.3DEE + 13.0DNFE
Emmans (1994)	NEee[1] (MJ/kg) = AME - 3.80FOM - 4.672DCP + 4.0DEE
Emmans (1994)	NEee[2] (MJ/kg) = 1.17AME - 4.2CP - 2.44

AME - Apparent Metabolisable Energy (MJ/kg);

DCP - digestive crude protein (kg/kg);

DEE - digestible total fat (Ether Extract) (kg/kg);

DNFE - digestible N-free extract (kg/kg);

DCF - digestible crude fibre (kg/kg);

CP - crude protein (kg/kg);

FOM - faecal organic matter (kg/kg);

[1] Correction coefficients for NEf calculation:

Disaccharide	- 0.63kJ/g;
Monosaccharide	- 1.26kJ/g;
Milk protein	+ 4.19kJ/g;
Milk fat	- 4.19kJ/g;

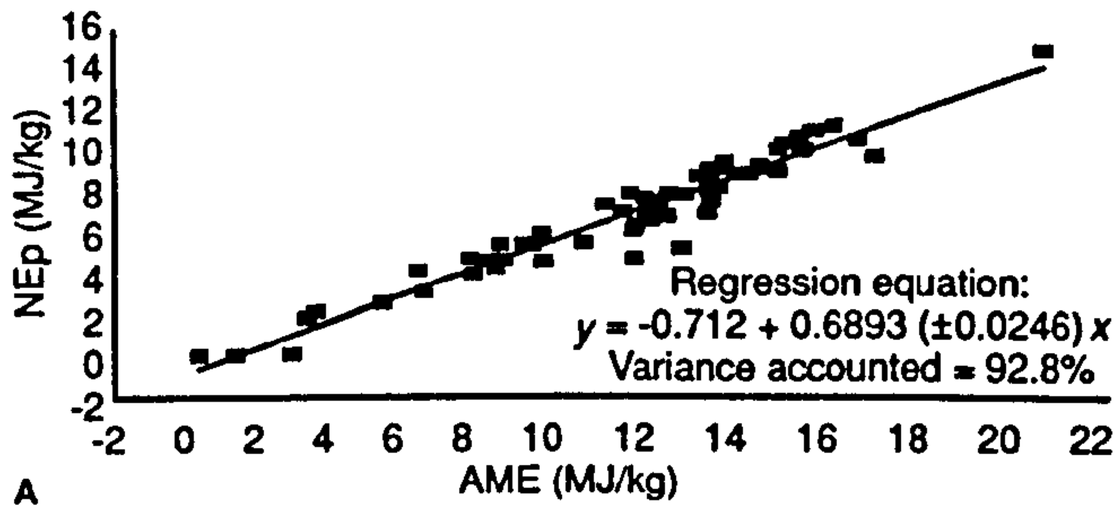
follows: Alfalfa meal 4%; Artichoke tuber 35%; hulless Barley 9%; Beans 16%; Cane seed 6%; Dried citrus pulp, 10%; Clover 10%; Dried collards 10%; Cottonseed hulls 11%; Wheat Flour (graham) 81%; Sorghum 26%; Grass (young) 44%; Rice hulls 4%. The correction coefficients for disaccharide, monosaccharide, milk fat and milk protein were not available to be used for the correction of the NEf values.

1. 4. 2. 2. Results and Discussion of quantitative review

There was a positive linear regression ($p<0.001$) of NEp on AME for the data set of 62 feedstuffs (Figure 1. 10). The standard error of the observations ((residual mean squares)$^{0.5}$) was 0.763MJ/kg. Each 1.0 MJ increase in AME resulted in a 0.69MJ increase in NEp. This relationship between AME and NEp is somewhat lower than a comparable estimate of the efficiency of nutrient use by growing poultry (De Groote, 1974a) and lower than the estimates of 0.78MJ in pigs (Noblet *et al.,* 1993). This confirms previously noted criticisms (Hill & Anderson, 1958, Davidson *et al.,* 1957) of the low NEp values obtained by Fraps. The negative intercept (-0.71) of the regression line was lower than that reported for growing pigs (-2.0) (Just 1982), due probably to the lower amount of fermentation that occurs in the digestive tracts of poultry.

The data subset of 40 feedstuffs not only represented the practical range used in modern broiler chicken feeds but also allowed an examination of whether feedstuff category affected the relationship between AME and NEp. There was evidence ($p<0.01$) that the AME determination overestimated the NEp of the animal

FIGURE 1. 10. RELATIONSHIP BETWEEN DETERMINED NET ENERGY FOR PRODUCTION (NEp) AND DETERMINED APPARENT METABOLISABLE ENERGY (AME):
(A) 62 feedstuffs with AME values of 0.1-20.5 MJ/kg.
(B) 40 feedstuffs with AME values of 8-16.5 MJ/kg.
(C) 40 feedstuffs with AME values of 8-16.5 MJ/kg in four categories.



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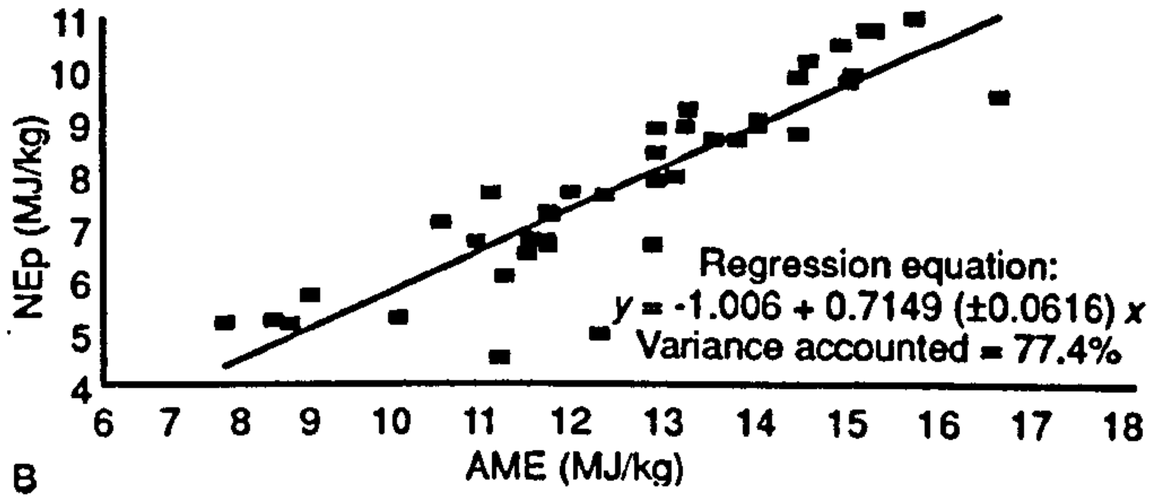

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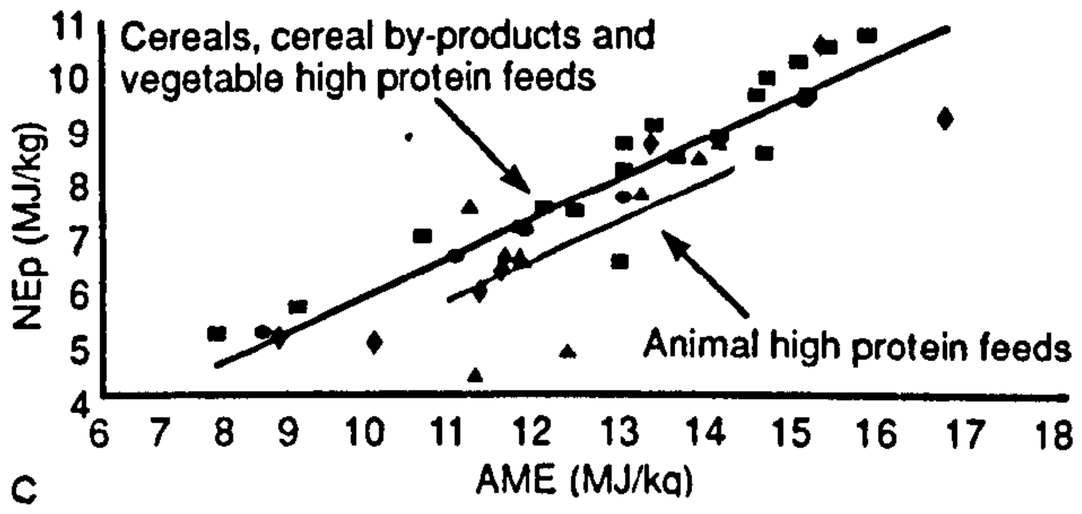


C

■, cereal and high starch foods; ●, cereal byproducts;
▲, animal high protein feeds; ◆, vegetable high protein feeds.

high protein feeds in comparison to the other three feedstuff classifications (Table 1. 13 and Figure 1. 12). There were no differences ($p>0.05$) between the regression coefficients determined separately for the four feedstuff groups but there were significant ($p<0.001$) differences in the intercepts of the four separate regression lines. Including the digestible protein concentration of the feedstuff with the determined AME in a multiple regression analysis improved the precision of the estimate of NEp and explained 84.2% of the variance. However, there was still statistical evidence that the response differed ($p=0.01$) among the four feedstuff groups.

The results from this statistical analysis show that there is a linear relationship between AME and NEp. However, the confidence intervals of the predicted NEp values were large relative to economically significant differences in available energy concentration between feedstuffs available to the poultry industry. For example, the 95% confidence interval of the mean NEp value (6.634MJ/kg) was $\pm$ 1.53MJ/kg. Although the high between-animal variability of the NEp determination may contribute to the poor precision (Carpenter, 1962), AME does not appear to be an accurate enough method of describing the variation in the NE of feedstuffs. Second, AME overestimates the NEp of animal high protein feeds and this problem could lead to nutritionists attributing false economic values to some feedstuff categories. A greater accuracy in estimating available energy concentration is required because of the high economic importance of available energy concentration in proprietary poultry feeds, so there is a need to examine other methods of estimating utilizable energy concentrations of poultry feeds.

TABLE 1. 13. RELATIONSHIP BETWEEN DETERMINED AME (independent variable) AND NEp (dependent variable) (n = 40) INCLUDING FEEDSTUFF CATEGORY AS A GROUPING FACTOR.

Dependent variable	Fitted terms in regression model	Regression coefficient (±SE)	Constant (±SE)	Standard error of observation	Percent variance accounted for
NEp (MJ/kg)	Constant + AME	0.7149	- 1.006	0.833	77.4
		(± 0.0616)	(± 0.761)		
NEp (MJ/kg)	Constant + AME	0.6866		0.761	81.1
	+ feedstuff	(0.0577)	Cereals: - 0.304		
			Vegetable proteins: -0.585		
			Animal proteis: - 1.276[1]		
			Cereal byproducts: - 0.899		
			(SE = 0.745)		

[1] Constant for the animal protein feeds was significantly ($p < 0.01$) lower than the constant for the cereals, vegetable proteins and cereal byproducts.

The regression analyses indicate that only three of the four NE prediction equations improved the precision in estimating the determined NEp of the 62 feedstuffs in comparison to using AME (Figure 1. 11). The standard errors of the observations were 0.812, 0.645, 0.662 and 0.687MJ/kg for NEf, NEue, NEee[1] and NEee[2] respectively compared with 0.763MJ/kg for AME. The low precision of the NEf calculation was surprising because of its similarity to the NEue equation. However, the NEf equation attributed a 19% lower energy contribution from digestible protein and 5% lower energy contribution from digestible fat compared to the NEue equation. The NEf equation also attributed a high NE contribution (13.4MJ/kg) to digestible crude fibre that was not included in NEue. A high number of the 62 feedstuffs had high crude fibre contents, with correspondingly low energy densities, and an inappropriate NE equation for this type of high fibre feedstuff was probably a major cause of the low precision. The regression analyses of the 40 sample data (feedstuffs with an AME between 8 and 16 MJ/kg) indicated that the precision of the NEf calculation was improved compared to no ($p>0.05$) change in the standard error of observations of the other three NE calculation methods (Figure 1. 12). The standard error of observations for the linear regression of each of the four NE calculations with determined NEp were 0.729, 0.672, 0.669, and 0.667 MJ/kg for NEf, NEue, NEee[1] and NEee[2] respectively compared with 0.833MJ/kg for AME.

The prediction of NEp using the NEee[2] equation gave similar standard errors as the NEee[1] equation. The similarity indicates that correction for the low efficiency of utilization of protein is the most important factor in adjustment of ME values to

FIGURE 1. 11. RELATIONSHIP BETWEEN DETERMINED NET ENERGY FOR PRODUCTION (NEp) (FRAPS, 1946) AND FOUR METHODS OF PREDICTION OF NET ENERGY: (A) NEf (Hoffmann & Shiemann, 1980); (B) NEue (DeCroote, 1974); (C) NEee [1] (Emmans, 1994); (D) NEee [2] (Emmans, 1994) OF 62 FEEDSTUFFS WITH AME VALUES OF 0.1 - 20.5 MJ/kg.

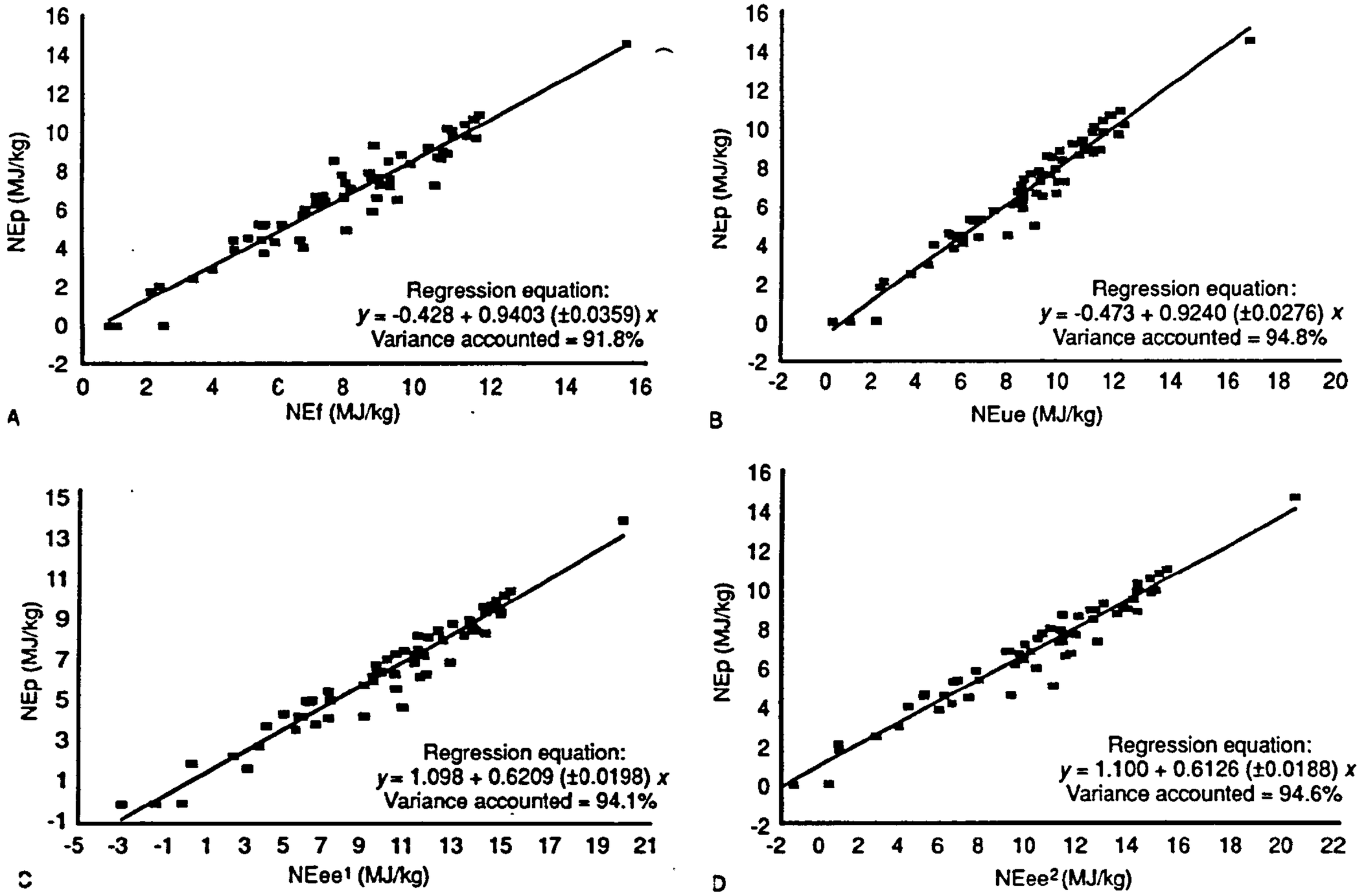
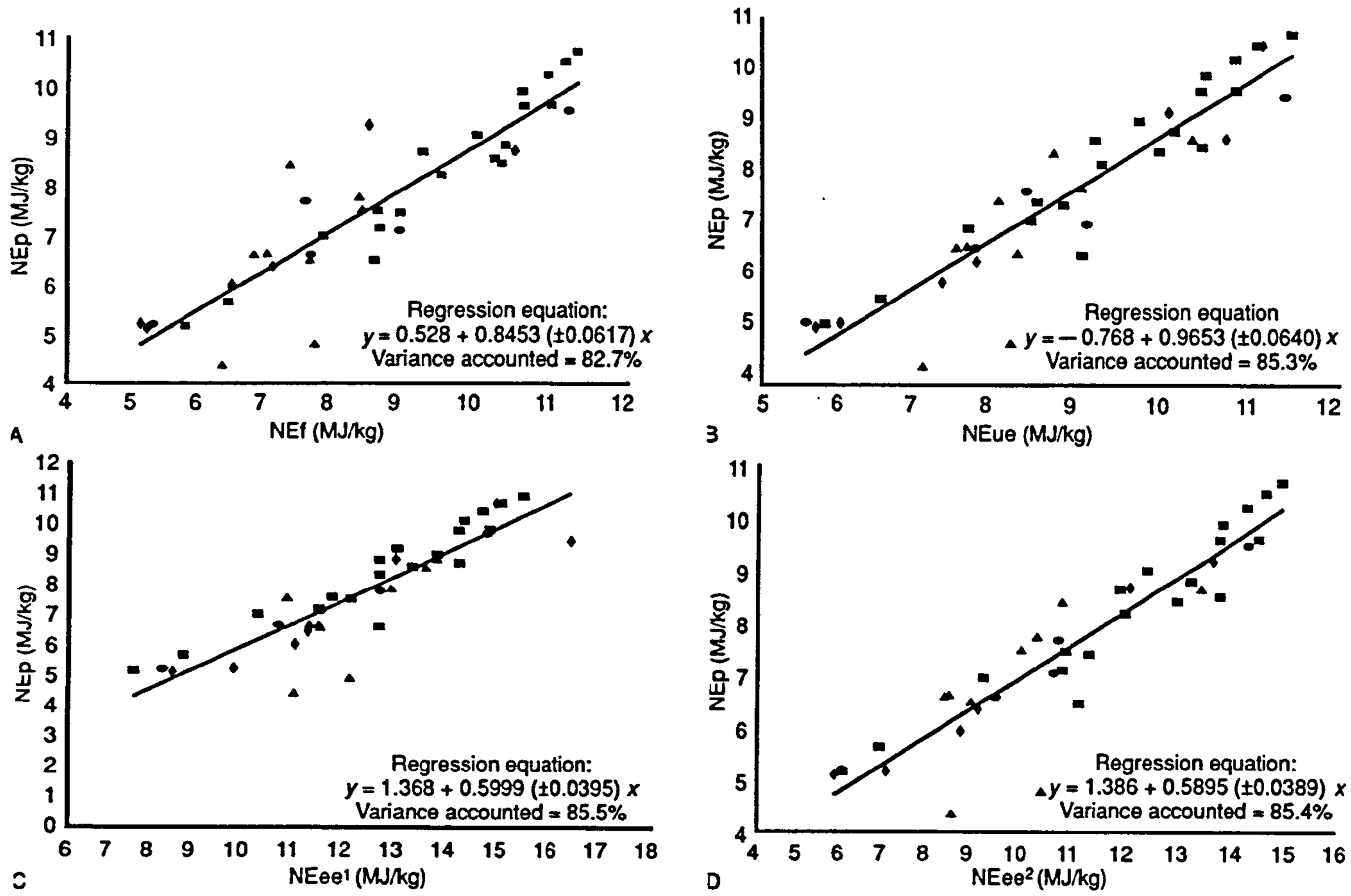


FIGURE 1. 12. RELATIONSHIP BETWEEN DETERMINED NET ENERGY FOR PRODUCTION (NEp) (FRAPS, 1946) AND FOUR METHODS OF PREDICTION OF NET ENERGY: (A) NEf (Hoffmann & Shiemann, 1980); (B) NEue (DeCroote, 1974); (C) NEee [1] (Emmans, 1994); (D) NEee [2] (Emmans, 1994) OF 40 FEEDSTUFFS WITH AME VALUES OF 8 - 16.5 MJ/kg.



■, cereals and high starch foods; ●, cereal byproducts; ▲, animal high protein feeds; ◆, vegetable high protein feeds

describe their NE. ME values are frequently determined to evaluate new feedstuffs and additionally determining the crude protein concentration of feedstuffs would be relatively quick and inexpensive.

All four NE prediction equations gave a numerically higher estimate of NE than the determined NEp. Each 1MJ/kg increase in NEf or NEue was associated with 0.93MJ/kg increase in NEp whereas each 1MJ/kg increase in both NEee calculations was associated with increases of 0.62MJ/kg in NEp. The two NEee calculations are based upon corrections to the determined AME, so the low efficiency of use of AME for NEp in the Fraps experiments would inevitably result in a higher estimate of NE by these prediction equations.

Regression analysis of the 40 feedstuff data set indicated that there was no evidence ($p>0.05$) that the precision of the estimate was improved by including feedstuff classification as a grouping factor in the regression analyses of any of these four prediction equations. The equations therefore give an improved method of estimating the utilizable energy concentration of poultry feeds in comparison to AME.

There is still a relatively large amount of unexplained variation in NEp when it is predicted by either of the four NE equations. Similarly, Just (1982) was only able to explain 90% of the variation in the determined NE of 40 pig feeds. The data produced by Fraps were from animal experimentation, so part of this unexplained variance will be due to the individual bird variation. However, there is also evidence

that the NE of poultry feeds vary due to factors not considered in any of the four prediction equations. For example, Collier *et al.*, (1995) fed six feeds that differed only in the sample of wheat and had similar fat, protein and starch concentrations. They found no significant differences in the determined AME but differences in the energy retention per MJ of ME intake of broilers given these feeds. Van der Meulen *et al.*, (1997) showed that there were differences in net utilizable glucose produced by *in vivo* digestive hydrolysis in pigs from two starches that had the same ileal digestibility. However, the starches differed in the amounts of lactate and volatile fatty acids produced in the small intestine, suggesting a difference in the extent of bacterial fermentation. Bacterial fermentation within the small intestine is one factor that may change the amount of utilizable energy derived from a feed which would not be detected by ME determinations nor by any of the four prediction equations. The effects of bacterial fermentation could be relatively large; Muramatsu *et al.*, (1994) observed a 16% increase in energy retention in germ free chickens given the same ME intake as conventional chickens.

Other dietary factors may also affect the efficiency of energy utilization by poultry. For example, Panja *et al.*, (1995) and Nitsan *et al.*, (1997) showed that the increases in energy retention of broilers fed diets with different added fats were not well correlated with the determined increase in metabolizable energy concentrations of the diets. The composition of the dietary fat may thus also affect the total amount of NE yielded from a diet.

In conclusion, this quantitative review has shown that, in comparison to

AME, NE prediction equations give improved estimates of the utilizable energy of feedstuffs. The improvement of precision is desirable because of the high economic importance of available energy concentration in proprietary poultry feeds. However, diet composition has effects on the yield of net energy that would not be detected by ME determination nor by the use of any of the four prediction equations. Future work that gives a greater understanding of the interactions of nutrients and their effects within the lumen of the digestive tract may enable more precise prediction equations for NE to be developed for poultry.

2. EXPERIMENTAL

2. 1. SECTION A. OVERALL OBJECTIVES

The major objectives of this part of the project were:

To examine the differences in the chemical composition, quality characteristics and energy content of twelve separate wheat cultivar samples. To examine whether there are differences in productive performance of broiler chickens when fed these wheat samples as part of nutritionally complete diets and whether these differences related to the laboratory measurements.

To examine whether there were differences between different wheat cultivar samples in the efficiency of utilization of ME as a source of NE. Then to examine the relationship between chemical composition of the wheat sample to the efficiency of utilization of ME.

2. 1. 1. Wheat samples

Twelve samples of winter wheat taken from two harvest years (1993, 1996) were used in broiler feeding experiments and laboratory analyses. Six cultivars were used from the 1993 growing season; Beaver, Brigadier, Dean, Rialto, Haven and Riband. Similarly, six cultivars were used from the 1996 season; Beaver, Brigadier, Dynamo, Hussar, Hunter and Riband. All the samples were produced on sites at

Harper Adams University College. The growing sites were split into three land blocks, with the six cultivars sown in a randomized design within each block. All the crops used in the project were treated with 160 kg N/ha. All samples were stored in bags at ambient air temperatures in a dry store. The wheat samples from 1993 were used after about four years of storage and the samples from 1996 after eight months of storage. Before the animal feeding experiments, wheat samples were hammer-milled using a 4mm screen and then mixed in a horizontal mixer with the other feed ingredients. Freshly milled wheat samples were used for each feeding experiment to avoid spoilage, and a sample was retained from each new batch of flour for laboratory analysis.

2. 1. 2. Laboratory analysis of the wheat samples. Materials and methods

The major chemical components and grain quality of the wheat samples were measured. Prior to analysis, fresh samples (100g) of the grains were ground in a laboratory cyclone sample mill to pass a 0.5 mm screen. All analyses were carried out in duplicate (or more replicates if needed) and are reported on a dry matter basis.

2. 1. 2. 1. Proximate analysis

Dry matter (DM) was determined by drying at 100°C for 24 h (AOAC 925. 10). Ash was measured in a muffle furnace at 500°C for 18 h. Crude protein (N x 6.25) in the samples was analyzed by the Kjeldahl method, using a Kjeltec 1035 Autoanalyzer (Perstorp Analytical, Hoganas, Sweden) (AOAC 984. 13). Oil (as

ether extract) was extracted with diethyl ether by the Soxhlet method (HT 1043 Extraction Unit, Perstorp Analytical, Hoganas, Sweden) (AOAC 920. 39). Gross energy (GE) of the diets and excreta was measured using a Parr adiabatic bomb calorimeter (Parr-1755, Parr Instruments Company, USA).

2. 1. 2. 2. Carbohydrate analysis

(i) Total starch, amylose and amylopectin

The content of total starch and amylose of the wheat samples was determined colorimetrically by a procedure devised by Gibson *et al.* (1997) (Amylose / amylopectin Assay Kit, Megazyme International Ireland Ltd., Ireland). The starch in the samples was completely dispersed by heating in dimethyl sulphoxide. Lipids were removed by precipitating the starch in ethanol and recovering the precipitated starch. After dissolution of the precipitated samples in an acetate-salt solution, amylopectin was specifically precipitated by addition of concanavalin A and removed by centrifugation. Then amylose, in an aliquot of the supernatant, was enzymatically hydrolysed to glucose. The total starch in a separate aliquot of the acetate-salt solution was similarly hydrolysed to glucose. Glucose oxidase-peroxidase reagent was added to both of the samples, and their absorbances compared against standard total starch absorbances. The amount of amylopectin was obtained as a difference between total starch and amylose. The method is summarised in Appendix 1.

All the colorimetric measurements were performed on a Beckman DU-640 Spectrophotometer (Beckman Instruments, Inc., USA).

(ii) Free sugars

Free sugars in the wheat samples were measured as described by Englyst *et al.* (1992) (Englyst Starch Kit, Dunn Nutrition Centre, Cambridge, UK). The starch in the samples was dispersed by heating (100° C water bath for 30min.) in 0.1 M acetate buffer. After cooling to room temperature, 1 ml of each sample or standard or blank was taken into a tube containing 2 ml absolute ethanol, and mixed. The tubes were centrifuged at 1500 g for 5 min, and 1 ml of the supernatant was removed into a test tube containing 5 ml of distilled water and mixed. The absorbance of the standards was measured in a spectrophotometer at 510 nm.

(iii) Non-starch polysaccharides

The content of non-starch polysaccharides (NSP) of each wheat cultivar was measured using the method proposed by Englyst and Cumming (1988) (Englyst Fiberzym Kit for Colorimetry, Dunn Nutrition Centre, Cambridge, UK). The procedure included an enzymatic-chemical method to separate the starch from the NSPs.

Starch (α-linked glucan) is easily hydrolysed with pancreatic amylase while NSPs (β-linked polymers) are totally resistant to hydrolysis by these digestive

enzymes. Starch in the wheat samples was completely gelatinized with dimethyl sulphoxide at 100° C and then removed by incubation with pancreatin and pullulanase (a debranching enzyme). For total NSP measurement, the starch-free residue was precipitated with ethanol and hydrolysed with sulphuric acid in ice-water. For insoluble NSP measurement, precipitation with ethanol was replaced by a 30 min extraction with phosphate buffer, pH 7, at 100°C. Sulphuric acid was used to hydrolyse the NSP portion to neutral sugars and uronic acid and the samples were measured by colorimetry at an absorbance of 530 nm. The amount of soluble NSP was obtained as a difference between total NSP and insoluble NSP.

The methodology is summarised in Appendix 2.

(iv) In vitro rate of starch digestion

In vitro rate of starch digestion (RSD) was determined as described by Waldron (1997) following the original method proposed by Englyst *et al.* (1992) (Englyst Starch Kit, Dunn Nutrition Centre, Cambridge, UK). The procedure simulated the physical and enzymatic actions in the gut. Each of the wheat samples was incubated in a shaking water bath in tubes containing marbles, physiological sodium acetate buffer, and hydrolytic starch enzymes (pepsin, pancreatin-amyloglucosidase-invertase mixture). The same amount of the aliquot (0.5 ml) were taken from the tubes at timed intervals (10, 25, 40, 60 and 120min) and the level of glucose was measured colorimetrically. The level of glucose from the timed intervals was plotted against time to produce an exponential rate curve ($y = a(1-e^{-cx})$) from which the rate constant (c) was determined.

The methodology is summarised in Appendix 3.

2. 1. 2. 3. Grain quality

Hagberg Falling Number (HFN) was measured (Hagberg, 1961; Perten, 1964) by Hagberg falling number apparatus model 1800 (Falling Number AB, Stockholm, Sweden) (AOAC 976.13). The alpha-amylase activity in wheat samples was measured using an air segmented flow autoanalyser (Skalar Ltd, York, UK). This system is based on a system described by Smith (1970) which utilizes the Farrand technique (Farrand, 1964) to measure *alpha*-amylase activity in a sample extract.

Endosperm hardness (EH) was determined according to AOAC method number 989.03 using an Oxford QN 1000 Near Infra-red Reflectance (NIR) Analyser (Oxford Instruments, Oxford, UK). One soft wheat sample was used as a zero calibration point. Specific density (kg/hl) of wheat samples was measured with a chondrometer ("Easy grain", Farm-Tec, UK).

To measure water extract viscosity (WV), the following procedure was used. The wheat sample (2 g) was soaked in a tube containing 4ml H_2O (40 °C water bath) for 30 minutes. The tube was centrifuged (10000g for 2 minutes), left for 15 minutes at room temperature, then a 0.5 ml aliquot was taken from the liquid portion in each of the tubes. The viscosity of this supernatant (in centipoise (cP) units) was measured using a rotating cone and cup viscometer (model DV - II + LV, Brookfield Engineering Laboratories, USA).

2. 1. 3. Apparent Metabolizable Energy and productive performance determination

The ME determinations involved feeding growing chickens nutritionally adequate diets that contained either 350 g/kg or 650 g/kg of each wheat sample (Table 2. 1). Metabolizable energy of each diet was determined by feeding ad libitum to growing chickens for 14 d with their food intakes recorded for the final four days and all excreta quantitatively collected over this period.

Female Cobb broiler chickens were obtained from a commercial hatchery at day old and were placed in a single floor pen and fed a proprietary broiler starter feed until 5 d of age. The birds were then placed in the experimental cages two days before the start of the feeding period to accustom them to a cage environment. On the first day of the experimental period (7 d age), the chicks were individually weighed and randomly placed in one of 48 cages with 0.3 m x 0.3 m wire floor area. Two birds were placed in each cage and the cages were arranged in four tier levels within a controlled environment room. Each of the 12 experimental diets (six wheat cultivars x 2 inclusion rates) were allocated at random to cages of birds in each of the four tier levels. The temperature was kept at 30 °C at 7d age and was gradually reduced to 26°C at the end of the 14 d feeding period. The light regimen was 23 h light and 1 h dark. Access to the feed and the water was ad libitum. Two time replicates were conducted for the 1996 harvest samples that resulted in data being obtained from eight replicate cages of birds for each of the twelve experimental

TABLE 2. 1. INGREDIENT COMPOSITION OF THE EXPERIMENTAL DIETS FOR COMPARING GROWTH PERFORMANCE AND AMEn.

Feedingstuff	35% test wheat + 65% basal diet	65% test wheat + 35% basal diet
Test wheat	350.0	650.0
Maize gluten meal	66.9	36.0
Hulless soya bean meal	74.3	40.0
Full fat soya	297.1	160.0
Fish meal	148.6	80.0
Lysine HCl	3.7	2.0
Methionine	3.7	2.0
Dicalcium phosphate	18.6	10.0
Vitamin mineral premix [1]	37.1	20.0
Total	1000	1000
Calculated analysis		
ME (MJ/kg)	12.8	12.9
Crude protein g/kg	327.7	227.2
Lysine g/kg	21.7	13.2
Methionine + cystine g/kg	14.8	9.6
Calcium g/kg	18.8	10.4
Phosphorus g/kg	10.9	7.4
Sodium g/kg	4.5	2.5

[1] The Vitamin Mineral Premix contained vitamins and trace elements to meet the requirements specified by NRC (1984). The major components were: phosphorus 95 g/kg, methionine 50 g/kg, calcium 219 g/kg, sodium 30 g/kg, copper sulphate 0.5 g/kg, selenium 10 mg/kg, retinol acetate 0.275 g/kg, cholecalciferol 625 mg/kg, alpha tocopherol 2.273 g/kg.

diets. Only one time replicate was conducted for the 1993 harvest samples so four replicate cages of birds were used.

All excreta were collected for the last four days of the feeding period and were immediately dried at 60°C. The gross energy of each dried excreta sample and the experimental diets were determined using an adiabatic bomb calorimeter (Parr Instrument Company, USA). Nitrogen contents were determined by the Kjehldahl procedure using a Kjeltec 1035 Autoanalyzer (Perstorp Analytical, Hoganas, Sweden). The AME of the twelve diets were calculated by deducting the amount of energy contained in the collected excreta output from the energy intakes of the birds over the four day period. The values were corrected to zero N-retention (34.4 MJ/kg N-retention, Hill & Anderson, 1958) and consequently expressed as AMEn. The AMEn concentrations of the six wheat cultivars were determined using an analysis of regression model (inclusion level of the basal feed as the explanatory variate) using parallel regression lines between wheat samples. The statistical significance of the difference in AMEn between the wheat cultivars was compared using a randomized block analysis of variance in which there was a factorial partitioning of the treatment sums of squares into six wheat cultivars x two levels of inclusion.

On the fifteenth day from beginning of the experiment the birds were weighed and killed by cervical dislocation. The contents of the digestive tract, from the bottom of the duodenal loop to Meckel's diverticulum, were immediately collected, centrifuged (10 000g for 2 min.) and viscosity was measured as described by Bedford and Classen (1992).

2. 1. 4. Net Energy determination

The net energy values of the wheat samples were determined using a comparative slaughter method modified from that described by Fraps (1946). Although Fraps described his procedure as 'Productive energy', it was a form of net energy determination: The carcass energy depositions of growing chickens fed a complete diet that included a test ingredient were compared with growing chickens fed the same diet with the test ingredient replaced by a known ingredient (usually maize). Adjustments for the different energy requirements for maintenance were made according to the different body weights of the birds. The modified protocol used in the present experiment gave rapidly growing broiler chickens a diet that was restricted to 90% of their daily ad libitum intake (determined in a previous study). The diet comprised a mixture of a basal feed and one of the six experimental wheat samples. This mixture formed a nutritionally complete diet (Table 2. 2). The proportions of the two components of the diet were such that the basal feed accounted for 50% of the predicted ad libitum intake and the wheat sample accounted for the remaining 40% of the predicted ad libitum intake. One treatment group was fed only the basal feed in a restricted amount that accounted for 50% of their predicted ad libitum intake. Although the basal feed was not a conventional broiler feed, it provided sufficient of all essential dietary nutrients except for a deficiency in energy. The 50% restriction of the basal feed still allowed a steady, but relatively slow, growth rate in these birds. The greater carcass energy retention of the 90% restriction group, in comparison to the 50% restriction group, was considered to be due to the additional wheat intake and so the net energy (for production) of the

TABLE 2. 2. INGREDIENT COMPOSITION OF THE NET ENERGY EXPERIMENTAL DIETS.

Feedingstuff	Basal diet	50 parts basal diet + 40 parts test wheat
Test wheat	-	444.4
Wheat	300	166.7
Maize gluten meal	33.3	18.5
Hulless soya bean meal	83.3	46.3
Full fat soya	433.3	240.7
Fish meal	83.3	46.3
Lysine HCl	3.33	1.85
Methionine	5.0	2.8
Dicalcium phoshate	25.0	13.9
Vitamin mineral premix [1]	33.33	18.51
Total	1000	1000
Calculated analysis		
ME(MJ/kg)	12.9	13.0
Crude protein g/kg	311.4	221.9
Lysine g/kg	21.0	13.2
Methionine + cystine g/kg	14.8	9.8
Calcium g/kg	17.5	10.0
Phoshorus g/kg	11.1	7.6
Sodium g/kg	3.6	2.1

[1] The Vitamin Mineral Premix contained vitamins and trace elements to meet the requirements specified by NRC (1984). The major components were: phosphorus 95 g/kg, methionine 50 g/kg, calcium 219 g/kg, sodium 30 g/kg, copper sulphate 0.5 g/kg, selenium 10 mg/kg, retinol acetate 0.275 g/kg, cholecalciferol 625 mg/kg, alpha tocopherol 2.273 g/kg.

individual wheat sample was calculated. The different growth rates of the 90% and 50% groups during the experiment would have resulted in different heat loss due to maintenance requirements. However, a theoretical calculation of this effect indicated that this difference was very small relative to differences in carcass energy deposition between the two groups. Hence no adjustment to the calculations was made to account for different maintenance requirements.

The described protocol was used to compare the net energy concentrations of the two batches of six wheat samples. A flock of female Cobb broiler chickens were obtained from a commercial hatchery at day old and placed in a single floor pen and fed a proprietary broiler starter feed until 5 d of age. Each of the six wheat samples from each growing season was added to the basal feed (40 parts wheat sample added to 50 parts basal feed). Seven dietary treatment groups were therefore compared (six 90% of ad libitum diets with different wheat samples plus one 50% of ad libitum basal feed). The experimental diets were randomized to appear once in each of 6 positional blocks. A second time replicate was conducted for each of the two sets of six wheat samples so a total of 12 cage replicates were used for each NE determination of each wheat sample.

The broiler chickens were placed in cages at 5 d of age and at 7 d of age they were weighed and the heaviest birds were retained. Two birds were placed at random into each of 42 cages. All dietary treatments were fed in restricted amounts. A daily feed allocation was placed into the feeder each 24 h. The total daily allocation of feed depended on the predicted ad libitum feed intakes of the birds but ranged from

29.7 g/b/d at the start of the feeding period to 72 g/b/d on the final day of the 14 d feeding period.

The birds were weighed and killed by cervical dislocation at the end of the feeding period. The whole carcasses of both birds from each cage were frozen and later minced. The minced carcasses of both birds were pooled, thoroughly mixed and sampled. The samples were freeze-dried then the content of gross energy, crude protein and ether extract was determined.

The net energy content of each of the wheat samples fed to each cage of birds was calculated using the following equation:

Equation 2.1. Net energy calculation:

$$NE\ (MJ/kg\ wheat)\ wheat = (E_{90} - E_{50}) / W$$

E_{90} – Total gross energy (MJ) content of chicken carcasses when fed the experimental diets with a 90% restriction;

E_{50} – Total gross energy (MJ) content of chicken carcasses when fed the basal diet with 50% restriction;

W – Amount (kg) of the experimental wheat sample included in diets fed under 90% restriction;

The efficiency of utilization of AMEn as NE was calculated by dividing NE by AMEn.

An additional treatment group was included in the experiment comparing the 1996 wheat samples. The purpose of this treatment group was to examine the difference in the carcass energy deposition when a test diet was given ad libitum instead of at 90% restriction. A combined wheat sample was prepared that included equal proportions of each of the 6 test 1996 wheat samples. This combined wheat sample was included at the same proportion in the diet as used for the individual wheat samples (40 parts wheat to 50 parts basal feed). The diet was fed ad libitum to the broiler chickens. The carcass energy contents of the birds were determined at the end of the feeding period and their feed intakes used to calculate their equivalent wheat and basal feed intakes. This treatment group had the same replication and otherwise the same bird husbandry as described for the individual wheat samples at 90% restriction and was randomized within the blocked experimental design.

2. 1. 5. True Metabolisable Energy determination

An adapted procedure (McNab & Blair, 1988) was used for nitrogen corrected true metabolisable energy determination. ISA brown cockerels, previously fed ad libitum, had all feed withdrawn but were allowed water ad libitum. The cockerels were kept in individual cages (0.6m x 0.7m floor area), at a constant house temperature 16°C and light regime 16 hours. All birds were given 50ml of a sucrose solution after 24 h of this period. After a further 24 h, 50g of the wheat sample was

intubated into the bird's crop. Each wheat sample was given to six birds and another twelve birds were used to estimate endogenous losses. These birds were fed 50ml sucrose solution. After a further 24 h, all birds were given 50ml water by tube. Excreta were collected for 48 h after feeding. The excreta were then dried at 60 °C before gross energy and nitrogen analysis.

2. 1. 6. Statistical procedures

Statistical analyses were performed using the Genstat V statistical software package (Genstat V release 3.22 for Windows, Lawes Agricultural Trust, 1995). The AMEn, NEp, TMEn concentrations of the experimental wheat samples and digesta viscosity were statistically compared using a randomized block analysis of variance.

Multiple linear regression analysis was used to assess the relationship between broiler growth performance and the chemical composition or grain quality of the wheat samples. The harvest year was taken as a grouping factor. A step-wise regression technique selected the terms to add as explanatory variables into a linear model. The three variables that described growth performance (growth rate, feed intake and feed conversion ratio) were used separately as the dependent variates. All chemical constituents of the wheat samples and all laboratory-based measures of quality were offered as terms in the multiple linear regression.

Similarly, linear regression analysis was used to assess the relationship between AMEn and NE of individual samples and the relationship between the efficiency of utilization of AMEn as a source of NE (NE/AMEn). Initially, the data were coded according to the harvest year and all regression analyses incorporated

these codes as grouping factors using parallel relationships between the two harvest years. However, there were no differences ($p > 0.05$) between the relationships in the two harvest year samples, so simple regression analyses are presented.

The coefficients of correlation were obtained for all chemical compositions and quality measurements of the wheat samples. Linear regression analysis was done between all the measurements obtained with the broilers as a response variate and growing years as an independent variate. To eliminate the differences between the years, the growth performance, viscosity and energy measurement data were substituted with the numbers of their standardized residuals.

2. 1. 7. Results

Proximate analysis and GE

There were differences in gross chemical composition and GE between the cultivars within the years (Table 2. 3). The amount of ether extract was more variable in 1996 wheats and the protein level varied more in 1993 wheat samples. On average, however, the samples from 1993 had a higher dry matter content than 1996 year samples.

Carbohydrates

The amount of total starch (TS) in the wheat samples ranged from 594 to 726 g/kg (DM) (Table 2. 4). The rate constant of *in vitro* rate of starch digestion

TABLE 2. 3. CHEMICAL COMPOSITION OF TWELVE WHEAT SAMPLES.

Wheat cultivar	Dry matter (g/kg)	Ash (g/kg DM)	Oil (g/kg DM)	Protein (g/kg DM)	GE (MJ/kg DM)
Year 1996					
Beaver	862	17.0	18.6	130.9	18.73
Brigadier	858	16.0	16.3	126.9	18.72
Dynamo	863	15.5	15.1	139.9	18.78
Hussar	857	16.1	16.3	124.4	18.61
Hunter	862	15.9	17.4	135.2	18.78
Riband	858	15.0	21.0	131.9	18.76
Year 1993					
Beaver	893	15.6	14.6	140.5	17.85
Brigadier	890	18.5	14.6	131.7	17.88
Dean	892	17.2	14.6	138.9	18.06
Rialto	896	18.8	15.6	151.2	18.16
Haven	895	19.0	16.8	138.2	17.99
Riband	889	15.3	15.7	131.4	18.05

TABLE 2. 4. CARBOHYDRATE ANALYSIS OF TWELVE WHEAT SAMPLES.

Wheat cultivar	Total starch (g/kg DM)	Amylose (g/kg DM)	Amylopectin (g/kg DM)	Amylose: Amylopectin ratio	Free glucose (g/kg DM)	Insoluble NSP (g/kg DM)	Soluble NSP (g/kg DM)	Total NSP (g/kg DM)	Rate of starch digestion (g/100g/min)
Year 1996									
Beaver	689	221	468	0.47	3.11	77.1	32.1	109.2	1.69
Brigadier	710	214	496	0.43	2.9	72.3	31.1	103.4	2.61
Dynamo	726	223	503	0.44	2.3	82.3	14.9	97.2	2.04
Hussar	716	227	489	0.46	3.82	79.1	49.1	128.2	2.46
Hunter	699	211	488	0.43	3.33	70.8	35.0	105.8	1.85
Riband	711	212	499	0.42	2.68	80.0	34.2	114.2	1.67
Year 1993									
Beaver	691	203	488	0.42	2.36	74.9	29.4	104.3	1.98
Brigadier	650	214	436	0.49	3.12	78.7	32.6	111.3	2.96
Dean	675	224	451	0.50	1.96	78.2	16.8	95.0	2.88
Rialto	594	209	385	0.54	1.66	84.5	42.1	126.6	2.98
Haven	676	199	477	0.42	3.36	75.1	18.1	93.2	2.5
Riband	702	207	495	0.42	2.13	74.6	17.6	92.2	2.36

measurement (RSD) varied between the cultivars from 1.69 to 2.98 g/100g/min. The level of free sugar ranged between 1.66 and 3.82 g/kg (DM) of the wheat sample. The mean NSP content of the wheat samples was 106.7 g/kg (DM) for total NSP, 77.3 g/kg (DM) for insoluble NSP and 29.46 g/kg (DM) for soluble NSP. The cultivars Hussar and Rialto had more total and soluble NSPs than other samples.

Grain quality

The α-amylase activity within the samples differed between the two harvest years (Table 2. 5). The mean activity was 83.7 mEU/g (DM) for 1996 and 34.6 mEU/g (DM) for 1993 samples. The average Hagberg falling number values were 334 and 443s for 1996 and 1993 year samples respectively. Cultivar Riband (1996 harvest year) had the lowest HFN and the cultivar Brigadier (1993 harvest year) had the highest HFN.

Endosperm hardness ranged between 10.3 and 76.7 relative units. Cultivar Beaver was the softest in both of the years, and Dynamo and Dean were the hardest wheats for 1996 and 1993 harvest years, respectively. The specific weight of the wheat samples was 72.6 kg/hl for 1996 and 80.5 kg/hl for the 1993 harvest year. The 1996 sample of cultivar Riband had the lowest specific weight, whereas the 1993 sample of Riband had the highest specific weight. The 1000 grain weight was higher for the 1993 wheat samples than the 1996 samples.

The water extract viscosity of wheat samples ranged between 2.0 and 5.6 cP.

TABLE 2. 5. QUALITY CHARACTERISTICS OF TWELVE WHEAT SAMPLES.

Wheat samples	Water extract viscosity (cP)	α -Amylase activity (mEU/g DM)	Hagberg falling number (s)	Endosperm hardness (relative units) (Range 0-100) (Soft-Hard)	Specific density (kg/hl)	Weight of 1000 grains (g DM)
Year 1996						
Beaver	3.7	108.6	305	10.3	73.1	45.6
Brigadier	2.6	99.0	310	55.4	74.1	47.2
Dynamo	2.0	41.0	400	67.6	72.9	46.3
Hussar	2.8	45.5	394	46.6	76.5	49.5
Hunter	4.0	120.9	332	23.7	72.4	44.6
Riband	2.2	87.4	264	34.0	66.4	45.1
Year 1993						
Beaver	5.3	49.0	369	19.7	79.6	53.8
Brigadier	3.2	21.1	541	57.8	80.2	57.9
Dean	2.6	32.0	473	76.7	80.6	59.3
Rialto	3.3	31.4	435	51.3	80.2	54.9
Haven	5.6	43.7	423	33.6	80.6	55.1
Riband	2.7	30.3	414	44.3	82.0	62.7

Growth performance and energy measurement

There was a relatively large range of weight gains (19%) and of feed intakes (13%), however these differences were not statistically significant ($p>0.05$) (Table 2. 6). The average values of jejunal digesta viscosity were 17.9 cP for 1996, and 11.8 cP for 1993 wheat samples (Table 2. 6). Only the differences in digesta viscosity for 1996 samples were statistically significant ($p<0.001$).

There was a significant ($p<0.001$) difference in TMEn between the cultivars Beaver and Riband in the 1996 samples and the TMEn of Riband similarly was greater than that of Beaver ($p<0.05$) in the 1993 samples. However, there were no significant differences ($p>0.05$) in the determined AMEn in either harvest year (Table 2. 6).

The experimental data for the determined NE concentrations of the wheat samples was more variable; the coefficients of variation of the AMEn and NE values were 2.3% and 6.6% respectively. However, there was a statistically significant ($p<0.001$) difference in NE between the cultivars Rialto and Brigadier in the 1993 samples and the NE of Hussar was greater than that of Beaver ($p<0.05$) in the 1996 samples.

Relationship between chemical composition of the wheat and chicken growth performance

The correlation coefficients were obtained using all the data from the laboratory analysis and broiler experiments (Appendix 4). The step-wise regression

TABLE 2. 6. THE GROWTH, FEED CONVERTION RATIO, VOLUNTARY FEED INTAKE AND DIGESTA VISCOSITY OF BROILERS FED DIETS CONTAINING 65% OF TWELVE WHEAT CULTIVARS AND THE DETERMINED AME, AMEn, TMEn AND NEp OF THE SAMPLES.

Wheat cultivar	Weight gain (kg/14 days)	Feed conversion ratio	Feed intake (kg/14 days)	Digesta viscosity (cP)	AME (MJ/kg DM)	AMEn (MJ/kg DM)	TMEn (MJ/kg DM)	NEp (MJ/kg DM)
Year 1996								
Beaver	0.483	1.500	0.723	20.1	15.42	14.39	14.79	7.05
Brigadier	0.487	1.489	0.723	16.2	15.56	14.51	15.40	7.81
Dynamo	0.505	1.445	0.729	10.6	15.48	14.41	15.36	8.07
Hussar	0.495	1.515	0.749	21.6	15.50	14.46	15.30	8.09
Hunter	0.450	1.509	0.673	30.7	15.56	14.51	15.65	7.78
Riband	0.483	1.477	0.713	8.6	15.58	14.40	15.84	7.99
SEM (DF)	0.01438 (34)	0.0273 (34)	0.01945 (34)	2.53*** (18)	0.336[1] (88)	0.283[1] (88)	0.117*** (28)	0.3337* (51)
Year 1993								
Beaver	0.473	1.652	0.781	12.25	14.72	13.98	14.96	7.26
Brigadier	0.430	1.666	0.717	7.69	14.78	14.11	15.31	7.96
Dean	0.532	1.544	0.821	15.76	14.87	14.15	15.36	7.66
Rialto	0.462	1.624	0.748	13.21	14.36	13.77	15.21	7.00
Haven	0.464	1.664	0.769	10.78	14.46	13.85	15.26	7.16
Riband	0.486	1.589	0.772	11.0	14.86	14.17	15.57	7.85
SEM (DF)	0.0252 (15)	0.0376 (15)	0.0366 (15)	1.926 (15)	0.339[1] (40)	0.280[1] (40)	0.1181* (29)	0.1415*** (55)

[1] Standard error of intercept.

*** $p<0.001$; * $p<0.05$.

technique identified the chemical components of the wheat samples, and the laboratory measures of quality, that minimised the residual mean squares for the growth performance variables of growth rate, feed intake and feed conversion ratio. For each of these three variables, the residual mean square was minimised by including only three terms in the multiple regresion equation (Table 2. 7). Total starch content and the amylose:amylopectin ratio of the starch were included as terms in the equations for all three variables. Hagberg falling number was included as the third term for weight gain and food intake whereas the concentration of free sugar in the wheat was included in the multiple regression equation for FCR.

2. 1. 8. Discussion

Chemical composition and quality measurements

The results of proximate nutrient and GE content of the twelve wheat samples were in a similar range to those measured in other studies (March & Biely, 1973; Coates *et al.*, 1977; McNab, 1991). The wheat samples from 1993 harvest year had a higher DM content and lower α-amylase activity, probably due to the longer period of storage. The amount of total starch, amylose:amylopectin ratio and NSP contents varied between the years but it was in a similar range to other reports (Rogel *et al.*, 1987; Annison, 1990; Nicol *et al.*, 1993; Gibson *et al.*, 1997; Waldron, 1997). The rate constants of *in vitro* starch digestion were similar to those reported by Waldron (1997).

TABLE 2. 7. THE RELATIONSHIP BETWEEN THE GROWTH PERFORMANCE OF BROILER CHICKENS FED DIFFERENT WHEAT CULTIVAR SAMPLES AND THE DETERMINED CHEMICAL COMPOSITION OF THE WHEAT SAMPLES.

Explanatory variates Response variate	Constant	Total starch (g/kg DM)	Amylose: amylopectin ratio	Third explanatory variate	Block effect	r^2	SEM
Feed intake (g/b)	0.228	0.000234			-0.0351	0.56	0.0143*
Feed intake (g/b)	-0.173	0.000606	0.333		-0.0451	0.71	0.0124*
Feed intake (g/b)	-0.419	0.000894	0.629	-0.0001871 HFN (s)	-0.0724	0.85	0.00935*
Weight gain (g/b)	0.224	0.000377			-0.0072	0.19	0.0263 NS
Weight gain (g/b)	-0.614	0.001152	0.695		-0.0281	0.54	0.0211 NS
Weight gain (g/b)	-1.049	0.001663	1.218	-0.000331 HFN (s)	-0.0764	0.78	0.0154*
FCR	1.823	-0.000301			-0.1208	0.79	0.0398***
FCR	2.917	-0.001313	-0.908		- 0.0936	0.86	0.0349***
FCR	2.598	-0.001176	-0.659	0.0461 free sugar (g/kg DM)	-0.1221	0.96	0.0190***

Statistical significance of regression equation: *** $p<0.001$; * $p<0.05$; NS $p>0.05$.

The wheat samples from the 1993 harvest year had a higher 1000 grain weight and higher specific weight compared to the 1996 samples. Overall, the results were similar to those reported by other authors (McNab, 1991; Waldron, 1997; Scott *et al.*, 1998).

Growth performance and energy measurement

The lack of statistical significance in the differences in growth performance was surprising because there was a large range of values between the chicks fed different wheat cultivars. The range of treatment differences in feed intakes were 13% and 10% for the 1993 and 1996 samples respectively. Similarly, the range of treatment differences in growth rates were 19% and 11% for the 1993 and 1996 samples respectively. Although these differences were not proven to be statistically significant, it is probable that they demonstrated consistent differences in nutritional value between the wheat cultivars. Waldron (1997) found significant differences ($p<0.001$) in broiler growth performance when comparing two wheat cultivars, Dean and Beaver. The 1993 samples in the present experiment included Dean and Beaver and the numerical differences were similar to those obtained by Waldron (1997). Collier *et al.* (1996) reported different FCRs ($p<0.05$) feeding six UK wheats and a similar ranking of the wheat cultivars were obtained in this experiment. The relatively high variability of the growth performance in the present experiments was unexpected as all attempts were made to reduce experimental error during the work. Unfortunately there was not enough wheat left to repeat the feeding trial to increase replication of the growth performance data.

The determined AMEn and TMEn of the wheat samples were in a similar range as reported in other studies of UK wheat samples (Davidson *et al.*, 1978; McNab, 1991; Waldron, 1997). There was also a small range in AMEn values in comparison to data from North American (Sibbald & Slinger, 1962; March & Biely, 1973) and Australian (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Annison, 1991) wheats.

Relationship between chemical composition of the wheat and chicken growth performance

The step-wise multiple regression analysis indicated that the content of total starch and the amylose:amylopectin ratio in the starch were the main predictors of growth, feed intake and FCR of the broilers. Increasing starch contents in the wheat cultivar samples gave increasing weight gain and feed intakes. Total starch in the wheat samples was negatively correlated with crude protein and NSPs. The experimental diets were formulated to supply a small excess of crude protein over that required by the broilers, so variation in this nutrient in the wheat samples would not be expected to give nutritional benefits. The NSPs may have provided an amount of available energy, however, because no exogenous xylanase enzymes were supplied in the experimental diets, the energy supplied would have been primarily a result of microbial fermentation. It is possible that energy supplied directly from starch enables broilers to have greater feed intakes and growth rates.

The relationship between the amylose:amylopectin content of the starch and growth performance of the chickens could have a number of explanations. First, it is

possible that there was a nutritional benefit in having a high total dietary supply of amylose. Different proportions of amylose and amylopectin could give a different release of mono-, di- and oligomers in the digestive tract (Moran, 1982): Digestion of amylose by α-amylase leads to maltose and maltotriose as the primary products, when amylopectin digestion releases an array of α-limit dextrins. Vohra and Kratzer (1964) noted that polysaccharides with branched structures tended to display a greater anti-nutritive activity. High amylopectin in contents in starch may release more oligosaccharides in the digestive tract and so depress the utilisation of the dietary energy. Reid *et al.* (1998) also noted that starches high in the amylopectin fraction are more accessible to bacterial fermentation after pancreatic enzyme treatment. The end products from microbial fermentation are much less efficiently utilised by pigs (Just *et al.*, 1983a,b) and presumably by chickens, compared to the utilization of glucose and lipid.

A second explanation of the relationship between amylose:amylopectin content and growth performance could be that starch granule structure is an important factor that affects broiler growth performance. Granules that have different proportions of amylose and amylopectin have different shapes and forms (See Section 1.2.2). The proportion of amylose and amylopectin in starch granules also affects granule integrity. Gallant *et al.* (1996) concluded that amylose content appears to be one of the factors involved in starch resistance to enzymatic and acid attack.

The step-wise multiple regression analysis indicated that the Hagberg falling number (HFN) was a third independent variable that predicted broiler growth and

feed intake. HFN is a measure of baking quality of wheat and is reciprocally related to the α-amylase activity. However, HFN is an empirical measurement and many other factors that affect the viscosity of the starch gel can increase the HFN of a wheat sample (Best & Muller, 1991). Wheat grains with high alpha-amylase activity result in lower HFN because the action of α-amylase caused increased liquefaction of the endospermic starch (by loss of gelatinisation properties due to hydrolysis of the available starch to maltose) which decreases the viscosity of the of the gelatinised wheat solution (Wiseman, 1990; Best & Muller, 1991). McNab (1991) found a strong negative relationship between the Hagberg number and TMEn in wheat. Rose *et al.* (1993) found the same trend between the Hagberg number and the AMEn and FCR of the broilers. Waldron (1997) also observed a negative correlation ($p<0.05$) between HFN and broiler FCR. None of these authors found a significant relationship between the Hagberg number and the α-amylase activity of the wheat. In the current study, increased HFN led to an increase of FCR ($p<0.001$) which is the opposite to previously published data. The reasons for this contradiction in results are unclear.

The step-wise multiple regression analysis indicated that the content of free glucose was important as a third independent predictor of broiler FCRs. An increased level of free sugar led to an increase in FCR ($p<0.001$). Waldron (1997) also found a negative relationship between amount of free sugar in wheat samples and the weight gain and feed intake of broilers. The amount of free sugars in both of the experiments was less than 10 g/kg wheat. Thus, it seems that the level of free sugars in the diets in current experiment was too low to have a direct nutritional

effect on the broilers. Sibbald and Price (1976) found a relationship between increased levels of soluble sugars and decreased available energy in damaged wheat samples. Batterham *et al.* (1976) concluded that there was a reduction in digestibility of both energy and crude protein in sprouted wheat samples. The amount of free sugar in the wheat may not be directly important but it is probably an indicator of previous enzymatic hydrolysis that occured prior to harvest or during storage.

Relationship between the efficiency of utilisation of AMEn as a source of NE and chemical and quality analysis of the wheat samples.

There was a significant ($p<0.05$) linear relationship between NE and AMEn (Figure 2. 1), although an r^2 of 0.42 indicated that there was a substantial amount of unexplained variation. The efficiency of utilization of AMEn for NE had a range of 0.49 to 0.56 in the 12 wheat samples. The relationship between the measured chemical composition of the wheat samples and the efficiency of utilization of AMEn for NE was tested. In the correlation analysis endosperm hardness was significantly correlated with NE/AMEn (Appendix 4), but in this analysis the year difference had been removed. The year difference in NE/AMEn was not significant, and therefore it was not removed in the step-wise regression analysis. There was a significant ($r^2=0.40$; $p<0.05$) linear relationship between the water-extract viscosity of the wheat sample (log transformed) and the efficiency of utilization of AMEn for NE (Figure 2. 2). There were no other significant ($p>0.05$) relationships between the chemical characteristics of the wheat samples and the efficiency of utilization of AMEn for NE.

FIGURE 2. 1. RELATIONSHIP BETWEEN NE AND AMEn FOR 1993 (O) AND 1996 (■) HARVEST YEAR SAMPLES.

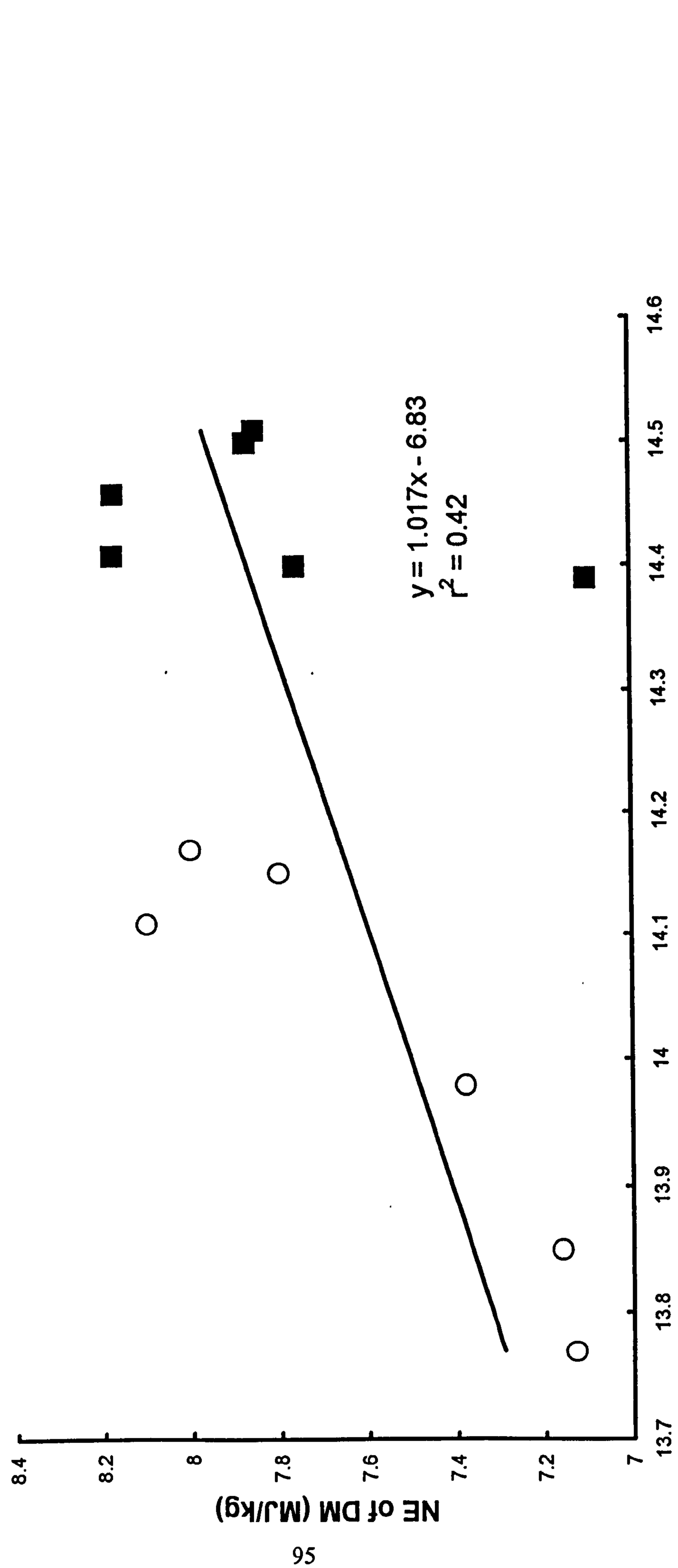
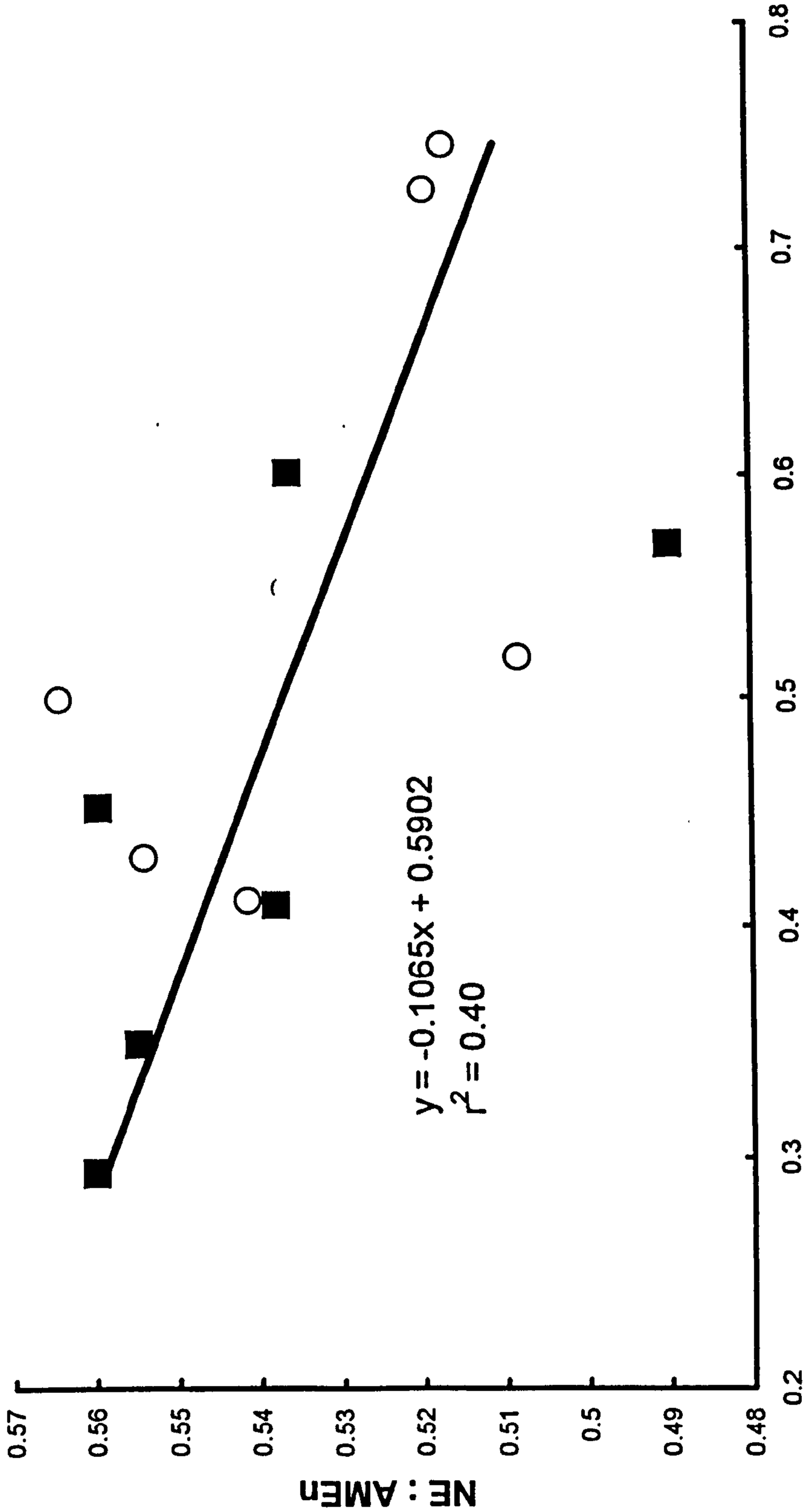


FIGURE 2. 2. RELATIONSHIP BETWEEN EFFICIENCY OF UTILIZATION OF AMEn FOR NE (NE:AAMEn) AND LOG WATER EXTRACT VISCOSITY FOR 1993 (O) AND 1996 (■) HARVEST YEAR SAMPLES.



The determined NE concentration of all the wheat cultivars was lower than expected. The determined efficiency of utilization of AMEn for NE was approximately 0.5 whereas Fraps (1946) found that the efficiency of utilization of ME in a number of different cereals was between 0.6 and 0.7. The birds given the combined 1996 wheat sample *ad libitum* had higher intakes of the test wheat sample but also the basal feed, thus it was not possible to derive an exact NE for the wheat sample. Although the AMEn of this mixture could not be exactly determined, it is probable that there was a higher efficiency of utilization of AMEn of approximately 0.6. The feed restriction method thus probably resulted in a lower determined overall NE value for the wheat samples. A comparison of the carcass composition of the birds given the *ad libitum* feeding showed that they had a greater ($p<0.001$) fat composition compared to the birds given the 90% feed restriction (Table 2. 8). Emmans (1994) proposed that the energy cost of carcass fat growth was less than that of carcass protein growth, particularly if dietary fat was used as the source of energy for the fat growth. It is possible that the low NE obtained in this experiment was due, at least in part, to the low carcass fat growth obtained in the 90% restricted chickens.

There was a relatively large range of determined NE values in the 12 wheat samples and a significant difference in NE between cultivars in both the 1993 and 1996 wheat samples. Although there was a linear relationship ($p<0.05$) between the determined AMEn and NE of individual wheat cultivar samples, there was still a large proportion of unexplained variation. One of the samples (Beaver 1996) (Table 2. 6) was an outlier to the general relationship between AMEn and NE. However, there was no obvious reason to eliminate this sample from the regression analysis

TABLE 2. 8. NET ENERGY (NE) DETERMINATION OF THE WHEAT SAMPLES. THE WHEAT INTAKES AND CARCASS COMPOSITION OF CHICKENS FED WHEAT BASED DIETS AND THE DETERMINED NE OF THE TWELVE WHEAT SAMPLES.

Feed	Feed allocation	Wheat intake of bird (kg of DM/bird)	Live weight of bird (kg/bird)	Total carcass dry matter (kg/bird)	Gross energy of carcass (MJ/kg DM)	Total carcass gross energy (MJ/bird)	Total carcass fat (g/bird)	Total carcass protein (g/bird)	Determined NE of wheat sample (MJ/kg DM)
Year 1996									
Basal feed	50% restrict		0.396	0.109	21.97	2.39	12	75	
Beaver	90% restrict	0.267	0.570	0.175	24.45	4.27	45	101	7.05
Brigadier	90% restrict	0.266	0.565	0.179	24.97	4.40	50	98	7.81
Dynamo	90% restrict	0.268	0.581	0.182	24.99	4.55	51	102	8.07
Hussar	90% restrict	0.269	0.588	0.184	24.83	4.51	50	103	8.09
Hunter	90% restrict	0.272	0.587	0.182	24.75	4.50	50	104	7.78
Riband	90% restrict	0.268	0.572	0.181	24.95	4.47	50	101	7.99
Mixture of all wheat samples	ad lib	0.307	0.657	0.213	25.61	5.46	70	114	-
SEM	-	0.0072	0.0010	0.0034	0.106	0.094	1.7	1.8	0.334
Year 1993									
Basal feed	50% restrict		0.381	0.106	22.45	2.39	15	72	-
Beaver	90% restrict	0.285	0.585	0.180	24.79	4.46	53	104	7.26
Brigadier	90% restrict	0.285	0.599	0.185	25.16	4.65	56	105	7.96
Dean	90% restrict	0.285	0.594	0.184	24.87	4.57	55	104	7.66
Rialto	90% restrict	0.286	0.589	0.178	24.60	4.43	51	105	7.0
Haven	90% restrict	0.283	0.574	0.177	24.90	4.41	54	99	7.16
Riband	90% restrict	0.284	0.603	0.185	24.96	4.61	55	106	7.85
SEM	-	0.0023	0.0046	0.0014	0.109	0.042	1.2	1.2	0.142

even though the r^2 value would have been increased considerably (from $r^2 = 0.42$ to $r^2 = 0.71$).

Wheat, like most other cereals, is used in poultry diets primarily because it is a low-cost source of available energy. Although wheat may provide up to 50% of the protein in a poultry feed, the protein is imbalanced and some essential amino acids have to be included in the diets (Pye, 1987). Therefore, the ME content of wheat is frequently used as the main characteristic that determines its economic worth when formulating practical poultry feeds. This experiment has shown that, although the ME of a wheat sample is relatively simple to obtain, there is variation in its efficiency of utilization in wheat-based practical diets. Thus, incorrect decisions may be made when assessing the economic worth of different wheat samples. The range of efficiencies of utilization of AMEn in this experiment were 0.49 to 0.56 and this could result in the feeding costs of a commercial broiler chicken flock differing by approximately 6%.

The variation in the efficiency of utilization of AMEn for NE could not be explained by differences in the proximate analyses of the wheat samples. However, there was a significant linear relationship ($p<0.05$) with the water-extract viscosity of the wheat samples. This characteristic, however explained only 40 % of the variation. Differences in water-extract viscosity could probably relate to differences in microbial proliferation and fermentation in the chicken's hind gut.

Microbial fermentation occurs, to some degree, in most parts of the chicken's digestive tract, and the extent of fermentation and the final products depend on the population size and microbe types within each part (Annison *et al.*, 1968). High levels of intestinal fermentation would probably increase the heat increment of

digestion and so reduce the net energy content of a feed and similarly reduce the efficiency of use of AMEn as a source of NE. Muramatsu *et al.* (1994) showed that conventional chickens had an increased heat production compared to germ-free chickens because of their increased microbial proliferation. Volatile fatty acids produced from the microbial fermentation of carbohydrates are poorly utilized by pigs (Just et al., 1983a,b) and presumably by chickens.

In conclusion, this part of the experiment has shown that there were differences in the net energy concentration of different wheat samples that were not related to their proximate nutrient compositions. Although the net energy concentration of a wheat sample was related ($p<0.05$) to its determined AME, there was still unexplained variation in the efficiency of utilization of AME as a source of NE. A proportion of this variation was explained by differences in the water-extract viscosity of the wheat samples.

2. 1. 9. General conclusion

The experiments on the twelve wheat samples have indicated that there was a significant relationship between the total starch and amylose:amylopectin ratio of the wheat samples and the resulting growth performance of chickens. There were differences in the efficiency of utilization of the ME between the wheat samples that were significantly related to the determined water-extract viscosity.

Both aspects of the study therefore gave commercially important results. Time limitations in the project meant that only one aspect could be investigated in

detail. The relationship between a chemical component of wheat samples and the growth performance of wheat is of great importance, not only to the UK broiler industry but also to UK cereal growers. It was therefore decided that the relationship between total starch and the amylose:amylopectin ratio and growth performance was most important. The remainder of the work reported in this thesis investigates this relationship.

2. 2. SECTION B. OVERALL OBJECTIVES

Results from the previous experiments indicated that increasing the level of total starch and amylose:amylopectin ratio in wheat improved growth performances in broiler chickens when fed these wheat samples as part of nutritionally complete diets. The overall objectives of the following experiments were, using an additional dietary maize starch and different types of cereals, to examine: a.) whether the improvements in broiler growth performance were due to the higher total dietary starch and amylose:amylopectin ratio that was provided in these diets, b.) whether there was an optimum amylose:amylopectin ratio which could be supplied by the diet or whether the effects observed in the previous experiments were specific to wheat starches only.

2. 2. 1. Nutritional comparison of the effect of different amount of additional maize starch varying in amylose:amylopectin ratio in wheat-based diet

2. 2. 1. 1. Introduction

Results from the previous experiments showed that variation in the amount of total starch and the amylose-amylopectin ratio of the starch were the most important predictors of the differences in growth performance of broilers fed different wheat cultivars. The range of starch contents of the twelve wheat cultivars was from 726 g/kg of DM to 594 g/kg of DM (cultivar Dynamo 1996 harvest year and cultivar

Rialto 1993 harvest year, respectively). Similarly, there was a range of 223:503 to 208:385 in amylose:amylopectin ratio between the wheat cultivars. There is a need to examine whether the improvements in broiler growth performance were due to the higher total dietary starch and/or amylose:amylopectin ratio that was provided in these diets. A number of options were examined to enable this experimental objective to be achieved.

i). *Obtain different wheat cultivars that had marked differences in starch amylose.*

Plant breeders in Japan and USA have recently begun to develop 'waxy' wheat cultivars (Yamamory *et al.*, 1995; Graybosch, 1998). These cultivars have a high proportion of amylopectin. One plant breeder (R. Graybosch, University of Nebraska, USA) was contacted. His selection program was in a relatively early stage and he was not able to provide a large enough sample for animal feeding studies. Small samples will be sent from the 1999 harvest to enable some preliminary animal feeding studies at a later date.

ii). *Separate amylose and amylopectin from wheat starch.*

Amylose and amylopectin are closely bound within the wheat starch granule so separation is technically difficult. ABR Foods Ltd, Corby, UK were contacted and confirmed that amylose separation is a current research area and that some success is

possible using a centrifugation technique. However, the amounts available from this process were not enough for animal feeding studies.

iii). *Obtain maize starch that had different amylose:amylopectin ratios.*

Maize cultivars have been selected for high level of amylose content. A high-amylose maize cultivar Hylon VII (70% amylose and 30% amylopectin) (National Starch & Chemical, UK) is available. A regular maize starch (25% amylose and 75% amylopectin) (National Starch & Chemical, UK) could thus be mixed with Hylon VII to give different amylose:amylopectin ratios.

The addition of the Hylon VII cultivar maize starch gave a practical method of changing the total dietary supply of starch with different amylose:amylopectin ratios. Although wheat and maize have different starch granule organizations there is no evidence that they behave differently as a source of dietary energy for poultry.

There is evidence (Ross & Mayhew, 1983; Langhout *et al.*, 1999) that the inclusion of some feedstuffs in diets can affect the growth performance, intestinal microbial activity and the morphology (shape of the villi) of the small intestinal wall in the poultry.

2. 2. 1. 2. Specific objectives

i). To examine the effect of three different levels of dietary starch on the growth performance and intestinal physiology and morphology of broiler chickens.

ii). To examine the effect of three different amylose:amylopectin ratios within each starch level.

iii). To examine the starch level x amylose:amylopectin ratio interactions.

iv). To examine single levels of two experimental starches (a chemically modified starch and a pure maize amylopectin) on the growth and digestive physiology variables.

2. 2. 1. 3. Materials and methods

Dietary treatments

A nutritionally complete basal feed was formulated (Table 2. 9). Three different levels of maize starch (20, 40 and 60 g/kg) were added to the basal feed in replacement of washed sand. Different combinations of two maize starches (conventional maize starch (250 g/kg amylose and 750 g/kg amylopectin) and high-amylose maize starch derived from Hylon VII (700 g/kg amylose and 300 g/kg amylopectin) (National Starch & Chemical, UK)) were used to obtain three amylose:amylopectin ratios (250:750, 475:525, 700:300) (Table 2. 10). Two further diets were formulated using either (a) modified wheat starch (Goldfield Sima) that

TABLE 2. 9. INGREDIENT COMPOSITION OF THE BASAL DIET IN FEEDING EXPERIMENT.

Ingredient	g
Wheat	650
Maize gluten meal	95
Hulless soya bean meal	32
Fish meal	100
Lysine HCl	5
Methionine	2
Soya oil	30
Dicalcium phosphate	6
Vitamin mineral premix [1]	20
Washed sand	60
Total	1000
Calculated analysis	
ME (MJ/kg)	12.7
Crude protein g/kg	220.0
Lysine g/kg	13.2
Methionine + cystine g/kg	10.0
Calcium g/kg	9.7
Phosphorus g/kg	6.4
Sodium g/kg	2.6

[1] The Vitamin mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1984). The major components were: Phosphorus 95 g/kg, methionine 50 g/kg, calcium 219 g/kg, sodium 30 g/kg, copper sulphate 0.5 g/kg, selenium 10 mg/kg, retinol acetate 0.275 g/kg, cholecalciferol 625 mg/kg, alpha tocopherol 2.273 g/kg.

TABLE 2. 10. PREMIX USED IN THE EXPERIMENT TO ACCOUNT FOR 60 g/kg OF THE COMPLETE DIETS.

Diet No	Washed sand (g)	Starch (g)	Components of added starch (g/kg)		
			Amylose	Amylopectin	Other
1	60	-	-	-	-
2	40	20[1]	250	750	-
3	20	40[1]	250	750	-
4	-	60[1]	250	750	-
5	40	20[1]	475	525	-
6	20	40[1]	475	525	-
7	-	60[1]	475	525	-
8	40	20[1]	700	300	-
9	20	40[1]	700	300	-
10	-	60[1]	700	300	-
11	-	60[2]	-	-	1000[2]
12	-	60[3]	-	1000	

[1] Mixed regular maize starch and high-amylose maize starch derived from the cultivar Hylon VII, National Starch & Chemical, UK; [2] Modified wheat starch behaved as amylose in an in vitro test (Goldfield Sima), ABR Food Ltd, UK; [3] Pure maize amylopectin, Fluka BioChemika, Switzerland.

behaved as amylose in an *in vitro* test (ABR Foods, Ltd, UK - personal communication), and (b) pure maize amylopectin (Fluka BioChemika, Switzerland).

Animal husbandry

The experiment was performed using broiler chickens between 7-21 days of age. Twelve experimental diets were randomized in 8 blocks of 96 cages. Two birds were placed in each cage and 8 replicates of each diet were used. Birds were kept in the same cages and conditions as in the apparent metabolisable energy experiments (See Section 2.1.3).

Measurements of jejunal viscosity, pH and villus shape

Jejunal viscosity. On the last day of the experiment the birds were killed by cervical dislocation. The contents of the digestive tract, from the bottom of the duodenal loop to the Meckel's diverticulum, were immediately collected, centrifuged and digesta viscosity measured as described previously (See Section 2.1.3).

pH of jejunal digesta. The pH was measured in the aqueous jejunal fraction that had been obtained for the jejunal digesta viscosity measurement. The measurement was taken by inserting a micro pH-electrode (Russel pH Ltd, Scotland) in the supernatant. Measurements were taken immediately after collection of the samples.

Morphological characteristics of the jejunal villi. Directly after collecting the digesta, samples from the mid-jejunal region of the intestines were taken and fixed in 3% glutaraldehyde solution from 10 randomly selected chickens from each diet. A section was taken at the antimesenteric attachment and fixed on cork plates with entomological pins. The shape of 50 villi from each intestinal sample was observed using a dissecting microscope (Kyowa, Japan) at 60x magnification. Each villus was classified into the following groups: tongue-shaped (Plate 2.1), finger-shaped (Plate 2.2) or conical-shaped (Plate 2.3).

2. 2. 1. 4. Statistical analysis

A randomized block analysis of variance was used to compare the treatment means. The treatment sums of squares of nine treatment means were partitioned to compare the main factor effects (level of starch and amylose:amylopectin ratio) and their interactions. Each of the two main treatment factor sums of squares were partitioned to identify the linear and non-linear effects. Specific orthogonal treatment comparisons were used to compare the effect of the three remaining treatment means. A chi-squared test was used to test the effect of diet on the shape of the jejunal villi.

2. 2. 1. 5. Results

i). Effect of the level of dietary starch.

PLATE 2. 1. LIGHT MICROGRAPH OF PART OF SECTION THROUGH THE JEJUNUM OF 21 DAYS OLD BROILER CHICKEN.

TONGUE-SHAPED VILLUS

PLATE 2. 2. LIGHT MICROGRAPH OF PART OF SECTION THROUGH THE JEJUNUM OF 21 DAYS OLD BROILER CHICKEN.

FINGER-SHAPED VILLUS

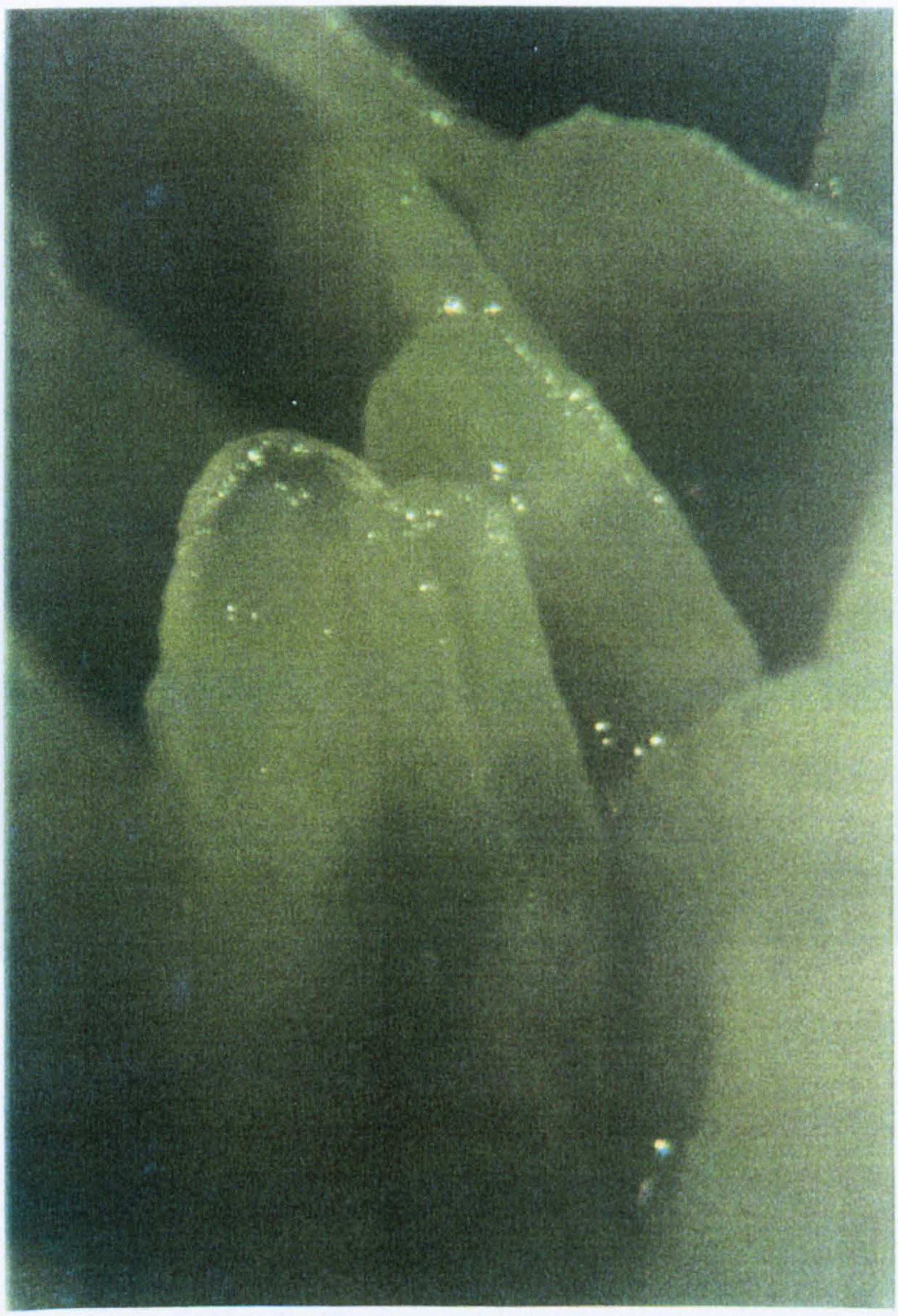
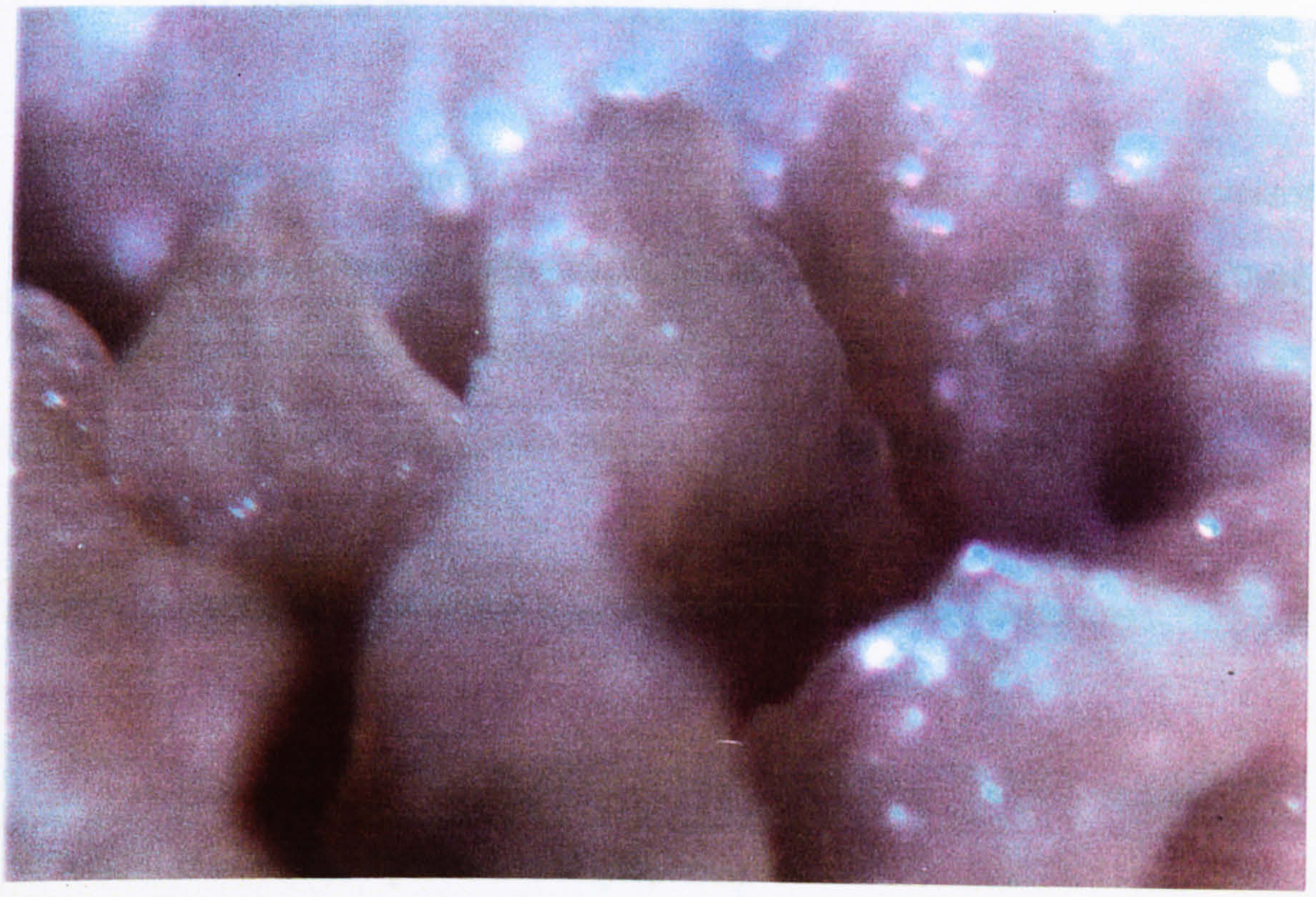


PLATE 2. 3. LIGHT MICROGRAPH OF PART OF SECTION THROUGH THE JEJUNUM OF 21 DAYS OLD BROILER CHICKEN.

CONICAL-SHAPED VILLUS



There was a negative linear relationship ($p=0.013$) between level of additional starch and FCR in broiler chickens (Table 2. 11). Every 10 g of additonal starch decreased FCR by 0.014. The increasing levels of starch did not affect weight gain, feed intake, jejunal viscosity or pH ($p>0.05$) (Table 2. 12). There were no interactions between the level of added starch and amylose:amylopectin ratio in the diets in any measured variables.

ii). Effect of the amylose:amylopectin ratio.

Increasing the amylose:amylopectin ratio in the additional starch increased weight gain and feed intake ($p=0.013$), but did not alter the FCR ($p<0.05$). There was no consistent effect on digesta viscosity or pH.

The amylose:amylopectin ratio of the diets affected the shape of the jejunal villi (Table 2. 13). The birds fed the high amylose:amylopectin ratios had a greater ($p<0.001$) proportion of conical shaped villi than the other two treatments.

iii). Effect of single levels of two experimental starches (a chemically modified starch and a pure maize amylopectin).

There were no significant differences ($p>0.05$) in growth performance or digesta pH and viscosity in the chickens fed the two diets (Table 2. 11).

TABLE 2. 11. THE EFFECT OF ADDED STARCH WITH DIFFERENT AMYLOSE:AMYLOPECTIN RATIOS ON GROWTH PERFORMANCE, JEJUNAL VISCOSITY AND JEJUNAL pH IN BROILERS FED DIFFERENT STARCHES FROM 7-21 DAYS OF AGE.

Diet No	Added starch	Weight gain (kg/bird)	Feed intake (kg/bird)	FCR	Jejunal viscosity (cP)	Jejunal pH
1	-	0.398	0.701	1.766	9.03	6.38
2	2% (250 g/kg amylose)	0.389	0.666	1.707	15.17	6.38
3	4% (250 g/kg amylose)	0.346	0.612	1.778	12.63	6.36
4	6% (250 g/kg amylose)	0.395	0.666	1.693	12.28	6.40
5	2% (475 g/kg amylose)	0.416	0.748	1.794	7.47	6.39
6	4% (475 g/kg amylose)	0.401	0.683	1.719	10.15	6.47
7	6% (475 g/kg amylose)	0.385	0.649	1.686	8.99	6.52
8	2% (700 g/kg amylose)	0.418	0.719	1.726	11.86	6.38
9	4% (700 g/kg amylose)	0.401	0.695	1.744	13.71	6.34
10	6% (700 g/kg amylose)	0.431	0.706	1.646	10.6	6.33
11	6% (modified starch)[1]	0.402	0.681	1.706	15.17	6.35
12	6% (1000 g/kg amylopectin)[2]	0.404	0.670	1.658	13.1	6.59
DF		63	63	63	63	63
SEM		0.0173	0.0255	0.0363	1.705	0.0691

[1] Modified wheat starch behaved as amylose in an in vitro test (Goldfield Sima), ABR Food Ltd, UK; [2] Pure maize amylopectin, Fluka BioChemika, Switzerland.

TABLE 2. 12. THE EFFECT OF DIFFERENT LEVELS ADDED STARCH WITH DIFFERENT AMYLOSE:AMYLOPECTIN (AM:AP) RATIO IN WHEAT-BASED DIETS ON GROWTH PERFORMANCE, JEJUNAL DIGESTA VISCOSITY AND JEJUNAL pH IN BROILER CHICKENS FROM 7-21 DAYS OF AGE.

2.12.1. Weight gain (kg/bird).

AM:AP ratio⇒ Added starch (g/kg diet)⇓	250:750	475:525	700:300	Effect of starch (p>0.05)	
20.0	0.390	0.391	0.418	**0.400**	SEM = 0.0111 (Effect of starch)
40.0	0.344	0.400	0.402	**0.382**	SEM = 0.0111 (Effect of AM:AP ratio)
60.0	0.394	0.385	0.431	**0.403**	SEM = 0.0192 (Effect of starch*AM:AP ratio) (p>0.05)
Effect of AM:AP ratio (p<0.05)	**0.376**	**0.392**	**0.417**		

2.12.2. Feed intake (kg/bird).

AM:AP ratio⇒ Added starch (g/kg diet)⇓	250:750	475:525	700:300	Effect of starch (p>0.05)	
20.0	0.672	0.711	0.719	**0.701**	SEM = 0.0161 (Effect of starch)
40.0	0.610	0.681	0.698	**0.663**	SEM = 0.0161 (Effect of AM:AP ratio)
60.0	0.666	0.649	0.706	**0.674**	SEM = 0.0279 (Effect of starch*AM:AP ratio) (p>0.05)
Effect of AM:AP ratio (p<0.05)	**0.649**	**0.680**	**0.708**		

2.12.3. Feed conversion ratio.

AM:AP ratio⇒ Added starch (g/kg diet)⇓	250:750	475:525	700:300	Effect of starch (p<0.05)	
20.0	1.714	1.830	1.726	**1.756**	SEM = 0.0222 (Effect of starch)
40.0	1.781	1.716	1.747	**1.748**	SEM = 0.0222 (Effect of AM:AP ratio)
60.0	1.694	1.686	1.646	**1.676**	SEM = 0.0384 (Effect of starch*AM:AP ratio) (p>0.05)
Effect of AM:AP ratio (p>0.05)	**1.730**	**1.744**	**1.706**		

2.12.4. Jejunal digesta viscosity (cP).

AM:AP ratio⇒ Added starch (g/kg diet)⇓	250:750	475:525	700:300	Effect of starch (p>0.05)	
20.0	16.38	10.85	11.86	**13.03**	SEM = 1.155 (Effect of starch)
40.0	12.63	10.15	13.57	**12.12**	SEM = 1.155 (Effect of AM:AP ratio)
60.0	12.28	8.99	10.60	**10.62**	SEM = 2.001 (Effect of starch*AM:AP ratio) (p>0.05)
Effect of AM:AP ratio (p>0.05)	**13.76**	**9.99**	**12.01**		

2.12.5. Jejunal pH.

AM:AP ratio⇒ Added starch (g/kg diet)⇓	250:750	475:525	700:300	Effect of starch (p>0.05)	
20.0	6.40	6.39	6.38	**6.39**	SEM = 0.0369 (Effect of starch)
40.0	6.36	6.47	6.35	**6.39**	SEM = 0.0369 (Effect of AM:AP ratio)
60.0	6.40	6.52	6.33	**6.42**	SEM = 0.0641 (Effect of starch*AM:AP ratio) (p>0.05)
Effect of AM:AP ratio (p>0.05)	**6.39**	**6.46**	**6.35**		

TABLE 2. 13. THE EFFECT OF AMYLOSE:AMYLOPECTIN RATIO IN ADDED STARCH ON SHAPE OF THE JEJUNAL VILLI IN BROILER CHICKENS AT 21 DAYS OF AGE.

AM:AP ratio ⇒				Σ =
Shape of the villi ⇓	250:750	475:525	700:300	
Conical-shaped	76	58	112	**246**
Finger-shaped	371	490	450	**1311**
Tongue-shaped	1053	943	947	**2943**
Σ =	**1500**	**1500**	**1500**	**4500**
$\chi^2 = 43.1$	$p < 0.001$			

2. 2. 1. 6. Discussion

Maize starch is a good source of available energy for poultry (McNab & Shannon, 1974) and the substitution of washed sand with starch gave a decreased ($p<0.05$) FCR but no effect on growth rate. However, in the previous wheat experiments, the starch content of the wheat was positively related to broiler growth and negatively related to FCR. The present experiment has indicated that the relationship between increased growth rate and starch content of a wheat sample was not due to the increased total starch supply in the wheat-based diets. It is possible that the apparent relationship with starch may have been due to a correlated variable. For example, there was a negative correlation ($r = - 0.41$) between total starch and nonsoluble NSPs in the wheat samples (Appendix 4).

The results of this experiment showed that increasing the amylose:amylopectin ratio in the additional starch increased growth performance. This is in agreement with the results obtained in the previous experiments (See Section 2.1.7). Different proportions of amylose and amylopectin could lead to different release of mono-, di- and oligomers in the digestive tract (Moran, 1982): Digestion of amylose by α-amylase leads to maltose and maltotriose as the primary products which additionally then includes an array of oligosaccharides (α-limit dextrins) when amylopectin is substrate. Some oligosaccharides have been shown to have a depressing effect on energy utilisation in poultry (Coon *et al.*, 1990). High amylopectin contents in starch may release more oligosaccharides in the digestive tract and so depress the utilisation of the dietary energy. The differences in villus

shape between the different amylose:amylopectin ratios in the experiment could therefore have been a consequence of these effects within the digestive tract.

The present experiment examined the addition of differing amounts of extracted starch from an unusual maize genotype (Hylon VII) that had selected for high amylose content that were specific only to this maize cultivar. It is possible, therefore, that the observed differences were relevant only to this starch source and not to an effect of the total amylose:amylopectin supply in the diet.

2. 2. 2. Nutritional comparison of the effect of additional maize starch varying in amylose:amylopectin content to three different cereal-based diets

2. 2. 2. 1. Introduction

Results from the previous experiment indicated that increasing the level of amylose in the diets improved growth performances in broiler chickens. It was not clear whether it was due to the total supply of amylose in the diets or just a specific effect of the additional amylose within the maize starch. As previously discussed, the best way to investigate the effect of amylose:amylopectin ratio in wheat is to make direct nutritional comparison between wheat cultivars with high and low amylose:amylopectin ratios. This experimental approach was not possible because of the current lack of a large enough sample for animal feeding studies. There is a need to examine the growth performance of broilers given diets with different total amylose and amylopectin supplies to examine whether there is an optimum amylose:amylopectin ratio or whether the effect observed in the previous experiment was specific to maize starches. There were some further options to produce different amylose:amylopectin ratios in broiler diets without relying solely on the addition of starch from the Hylon VII maize cultivar.

i). *Low and high amylose barley cultivars*

There are available some high- and low-amylose barley cultivars (Bergh *et al.*, 1999). Barley has a high starch content but, compared with wheat, it contains

more NSPs, particularly β-glucans (See Section 2.1.2.2) which could affect broiler growth performance.

ii). *High amylose rice cultivars*

Rice has a high content of highly digestible starch and a low amount of dietary fibre (Panigrahi *et al.*, 1992). There are available some rice cultivars with comparatively large differences in their amylose:amylopectin ratio. One rice supplier (Bruno Santini & C.s.a.s., Italy) was contacted. Rice (long-grain cultivar Thaibonnet, Italy) with a high level of amylose was supplied. In comparison, a sample of short-grain rice (unknown cultivar) was obtained that had a conventional amylose:amylopectin ratio.

2. 2. 2. 2. Specific objectives

The main objectives of the current experiment were:

i). To compare the growth performance and digestive physiology of growing chickens fed three cereals (two rice samples that differed in the amylose:amylopectin ratio of the starch and one wheat sample).

ii). To compare these variables in growing chickens fed four different amylose:amylopectin ratios (provided by supplementary maize starch) within each of the three cereal diets.

iii). To examine the cereal type x maize amylose:amylopectin ratio interaction.

2. 2. 2. 3. Materials and methods

Chemical analysis of the cereals

To measure chemical composition of the three cereals, freshly milled samples were used.

Dry matter, crude protein and ether extract concentrations were determined according to AOAC (1990) procedure numbers 925.10, 984.13 and 920.39 respectively.

Total starch, amylose and amylopectin contents of the wheat samples were determined colorimetrically by a procedure devised by Gibson *et al.* (1997) (Amylose / Amylopectin Assay Kit, Megazyme International Ireland Ltd., Ireland) (See Section 2.1.2.2).

Non-starch polysaccharides (NSP) of wheat were measured using a colorimetric method (Englyst & Cumming, 1988) (Englyst Fiberzym Kit for Colorimetry, Dunn Nutrition Centre, Cambridge, UK) as described before (See Section. 2.1.2.2).

Wheat had a markedly different chemical composition compared to the rice cultivars, and it contained more protein, ether extract and NSPs and less starch (Table 2. 14). Cultivar Thaibonnet contained more amylose (172g/kg vs 147g/kg), a higher amylose:amylopectin ratio and less ether extract and starch than the other rice

TABLE 2. 14. CHEMICAL COMPOSITION OF THE CEREALS (AS FED) USED FOR DIET FORMULATION IN FEEDING EXPERIMENT.

Chemical composition ⇒ Cereal ⇓	Dry matter (g/kg)	Crude protein (g/kg)	Ether extract (g/kg)	Total starch (g/kg)	Amylose (g/kg)	Amylopectin (g/kg)	Amylose: amylopectin ratio	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	Total NSP (g/kg)
Wheat	862	113	12.6	594	190	405	0.469	23.6	76.5	100.1
Long-grain rice (Cultivar Thaibonnet)	876	73	3.0	715	172	543	0.317	7.3	8.8	16.1
Short-grain rice (Unknown cultivar)	860	73	4.6	747	147	600	0.245	7.4	8.3	15.7

sample. The amounts of protein and NSPs were similar. The rice samples had chemical compositions similar to other rice samples as described by Panigrahi *et al.* (1992).

Dietary treatments

A practical broiler starter feed formulation was designed that included 650 g/kg of the test cereals (Table 2. 15). The three cereals provided different levels of all nutrients but it was considered that the relatively small variations in amino acids, minerals and vitamins would not affect growth performance or digestive physiology. The diet formulation also included 60 g/kg of maize starch (Table 2. 16). Combinations of two maize starches (conventional maize starch (30% amylose) and high-amylose maize starch derived from the Hylon VII cultivar (70% amylose) (National Starch & Chemical, UK) were used to obtain four amylose:amylopectin ratios in the added maize starch (250:750, 385:615, 565:425 and 700:300).

Animal husbandry

The experiment was performed with female Cobb broiler chickens between 7-21 days of age. Twelve experimental diets were randomized in 8 blocks of 96 cages. Two birds were located in each cage and 8 cage replicates of each diet were used. Birds were kept in the same conditions as in the growing experiments (See Section 2.1.3).

TABLE 2. 15. INGREDIENT COMPOSITION OF THE DIETS IN FEEDING EXPERIMENT.

Ingredient	Experimental diets (kg/tone)
Experimental cereal sample	650
Experimental maize starch	60
Maize gluten meal	95
Hulless soya bean meal	32
Fish meal	100
Lysine HCl	5
Methionine	2
Soya oil	30
Dicalcium phosphate	6
Vitamin mineral premix [1]	20
Total	1000

Calculated analysis	Wheat-based diets	Rice-based diets (Long grain rice)	Rice-based diets (Short grain rice)
ME (MJ/kg)	13.7	13.8	13.8
Crude protein g/kg	218.4	192.4	192.4
Lysine g/kg	13.1	12.6	12.6
Methionine + cystine g/kg	10	10.1	10.1
Calcium g/kg	9.8	9.9	9.9
Phosphorus g/kg	6.4	5.5	5.5
Sodium g/kg	1.2	1.6	1.6
Total starch	594	715	747

[1] The Vitamin Mineral Premix contained vitamins and trace elements to meet the requirements specified by NRC (1984). The major components were: phosphorus 95 g/kg, methionine 50 g/kg, calcium 219 g/kg, sodium 30 g/kg, copper sulphate 0.5 g/kg, selenium 10 mg/kg, retinol acetate 0.275 g/kg, cholecalciferol 625 mg/kg, alpha tocopherol 2.273 g/kg.

TABLE 2. 16. THE AMOUNT OF STARCH AND AMYLOSE:AMYLOPECTIN RATIO IN THE DIETS USED IN FEEDING EXPERIMENT.

Diet No	Cereal	Total starch in the diets (g/kg)	Amylose in added starch (g/kg)	Amylopectin in added starch (g/kg)	Amylose: amylopectin ratio in added starch	Amylose in the diets (g/kg)	Amylopectin in the diets (g/kg)	Amylose: amylopectin ratio in the diets
1	Wheat	493	250	750	0.333	146	347	0.421
2	Wheat	493	385	615	0.626	154	339	0.454
3	Wheat	493	565	425	1.329	165	328	0.503
4	Wheat	493	700	300	2.333	173	320	0.541
5	Long-grain rice	577	250	750	0.333	132	445	0.297
6	Long-grain rice	577	385	615	0.626	140	437	0.320
7	Long-grain rice	577	565	425	1.329	151	426	0.354
8	Long-grain rice	577	700	300	2.333	159	418	0.380
9	Short-grain rice	598	250	750	0.333	115	483	0.238
10	Short-grain rice	598	385	615	0.626	123	475	0.259
11	Short-grain rice	598	565	425	1.329	134	464	0.289
12	Short-grain rice	598	700	300	2.333	142	456	0.311

Digesta characteristics

Jejunal viscosity. On the last day of the experiment (21 days of age) the birds were weighed and killed by cervical dislocation. The contents of the digestive tract, from the bottom of the duodenal loop to the Meckel's diverticulum, were immediately collected, centrifuged (10000g for 2 min.) and jejunal viscosity measured as described before (See Section 2.1.3).

pH of jejunal digesta. A jejunal aqueous fraction was obtained for the jejunal digesta viscosity measurement. The pH of this fraction was measured using a pH-micro electrode as described before in the previous experiment (See Section 2.2.1.3).

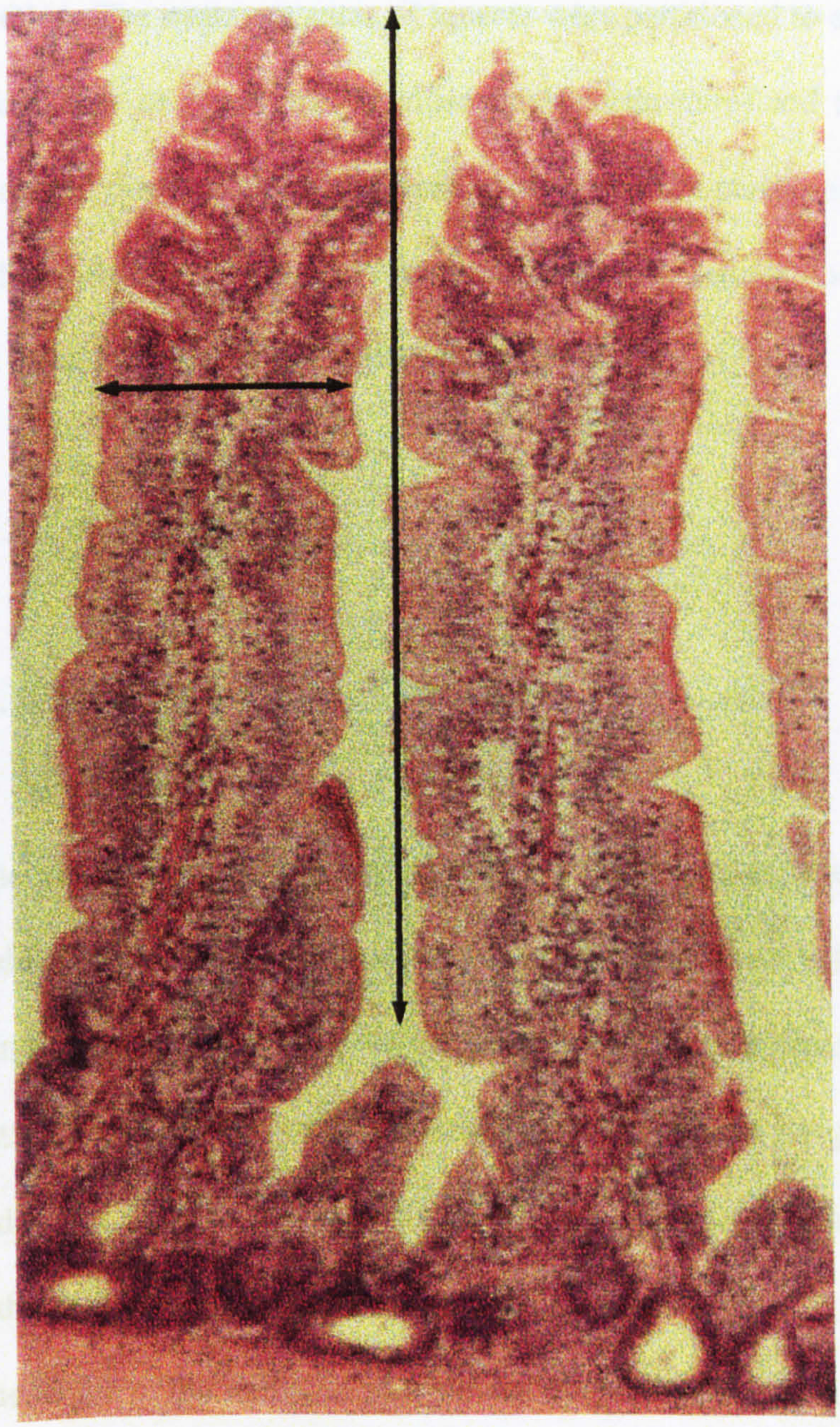
Jejunal morphometric studies

Only six dietary treatments were selected for these measurements. These were treatments with the most pronounced differences in growth performances and comprised the 250:750 and 385:615 amylose:amylopectin ratios in each of the three cereal types. Jejunal samples were taken from ten birds from each diet (selected at random). Cross-sections of the mid jejunum were embedded in paraffin wax and stained with haematoxylin and eosin for quantifying goblet cells. Morphometric measurements used a light microscope (Olympus, Japan) under 100x magnification. The height of the villus was represented by the distance from the crypt opening to the tip on the right side of the villus, as explained by Sigleo *et al.* (1984) (Plate 2. 4). Villus thickness was measured at a point one-third from the villus tip. The number of

PLATE 2. 4. LIGHT MICROGRAPH OF PART OF SECTION THROUGH THE JEJUNUM OF 21 DAYS OLD BROILER CHICKEN.

THE VERTICAL LINE SHOWS THE MEASUREMENT OF VILLUS LENGTH (CRYPT TO TIP)

THE HORIZONTAL LINE SHOWS THE MEASUREMENT OF VILLUS THICKNESS



total goblet cells per villus was determined by counting at 400x magnification, using 30 villi of each group of chickens (Plate 2. 5).

2. 2. 2. 4. Statistical analysis

Results from the experiment were statistically compared using a randomized block ANOVA. The treatment sums of squares were partitioned to compare the main factor effect (type of cereal and amylose:amylopectin ratio) and their interactions. The amylose:amylopectin treatment sums of squares were partitioned to identify the linear and non-linear effects. The treatment effect on jejunal morphometry were compared using a 2 x 2 factorial treatment structure.

2. 2. 2. 5. Results

i). Effect of cereal type

The growth performance and digesta viscosity were affected by the type of cereal (Table 2. 17). Wheat based diets gave higher weight gains, feed intakes, feed conversion ratios and jejunal digesta viscosity ($p<0.001$) compared to the two rice-based diets. Chicks fed the lower amylose rice had a higher weight gain and feed intake and lower digesta viscosity ($p<0.001$) than those fed the high-amylose rice although the FCR and jejunal pH were not significantly different.

The length of the villi were in a similar range to those measured by Langhout *et al.* (1999) (Table 2. 18). Cereal type affected the chicken's jejunal morphometry

PLATE 2. 5. LIGHT MICROGRAPH OF PART OF SECTION THROUGH THE JEJUNUM OF 21 DAYS OLD BROILER CHICKEN.

THE ARROW SHOWS THE GOBLET CELLS

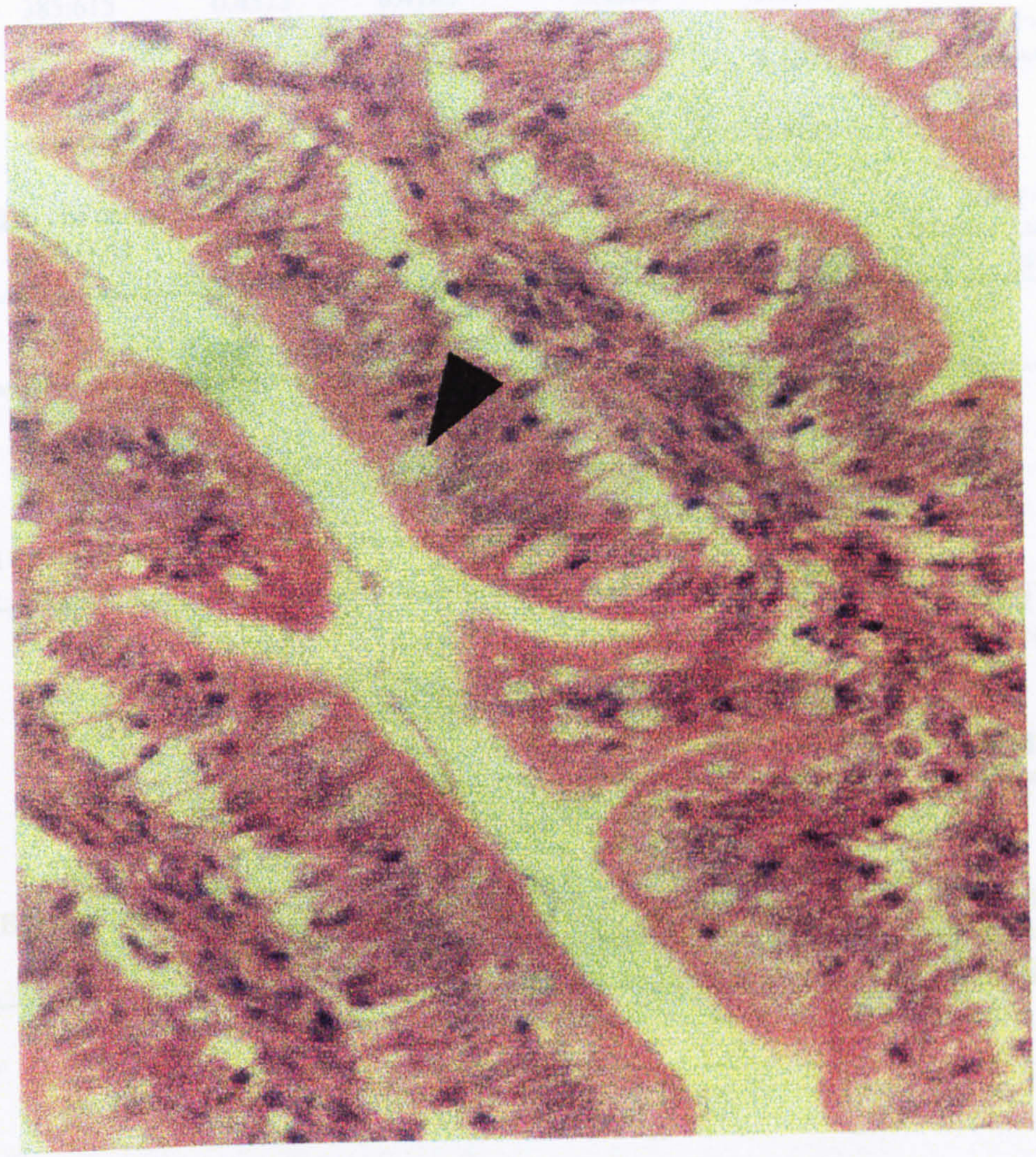


TABLE 2. 17. THE EFFECT OF CEREAL TYPE AND AMYLOSE:AMYLOPECTIN (AM:AP) RATIO ON GROWTH PERFORMANCE, JEJUNAL DIGESTA VISCOSITY AND JEJUNAL pH IN CHICKEN BROILERS FROM 7-21 DAYS OF AGE.

2.17.1. Weight gain (kg/bird).

AM:AP ratio in maize starch ⇓	Cereal type: Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio ($p<0.05$)	
250:750	0.4906	0.4393	0.4850	**0.4717**	SEM = 0.01014 (Effect of AM:AP ratio)
385:615	0.4572	0.4183	0.4368	**0.4374**	
565:425	0.4901	0.4479	0.4568	**0.4649**	SEM = 0.00878 (Effect of cereal)
700:300	0.5047	0.4258	0.4931	**0.4745**	
Effect of cereal ($p<0.001$)	**0.4856**	**0.4329**	**0.4679**		SEM = 0.01756 (Effect of AM:AP ratio*cereal) ($p>0.05$)

2.17.2. Feed intake (kg/bird).

AM:AP ratio in maize starch ⇓	Cereal type: Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio ($p>0.05$)	
250:750	0.7480	0.6261	0.7048	**0.6929**	SEM = 0.01347 (Effect of AM:AP ratio)
385:615	0.7341	0.6420	0.6622	**0.6794**	
565:425	0.7613	0.6516	0.6771	**0.6967**	SEM = 0.01167 (Effect of cereal)
700:300	0.7663	0.6749	0.7290	**0.7234**	
Effect of cereal ($p<0.001$)	**0.7524**	**0.6486**	**0.6933**		SEM = 0.02334 (Effect of AM:AP ratio*cereal) ($p>0.05$)

2.17.3. Feed conversion ratio.

AM:AP ratio in maize starch ⇓	Cereal type: Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio (p<0.001)	
250:750	1.5260	1.4346	1.4559	**1.4722**	SEM = 0.01471 (Effect of AM:AP ratio)
385:615	1.6174	1.5361	1.5184	**1.5573**	
565:425	1.5565	1.4554	1.4831	**1.4983**	SEM = 0.01274 (Effect of cereal)
700:300	1.5189	1.5897	1.4810	**1.5298**	
Effect of cereal (p<0.001)	**1.5547**	**1.5039**	**1.4846**		SEM = 0.02548 (Effect of AM:AP ratio*cereal) (p<0.05)

2.17.4. [1] Jejunal digesta viscosity (cP).

AM:AP ratio in maize starch ⇓	Cereal type: Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio (p>0.05)	
250:750	18.24	1.99	2.03	**7.42**	SEM = 0.028 (Effect of AM:AP ratio)
385:615	21.30	2.76	2.29	**8.78**	
565:425	17.79	2.38	2.25	**7.47**	SEM = 0.024 (Effect of cereal)
700:300	16.34	2.47	2.41	**7.07**	
Effect of cereal (p<0.001)	**18.42**	**2.40**	**2.24**		SEM = 0.048 (Effect of AM:AP ratio*cereal) (p>0.05)

[1] The **SEM** levels were obtained from log viscosity data analysis.

2.17.5. pH in chicken jejunum.

AM:AP ratio in maize starch ⇓	Cereal type: Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio (p>0.05)	
250:750	6.106	6.024	6.238	**6.123**	SEM = 0.0403 (Effect of AM:AP ratio)
385:615	6.123	6.024	6.142	**6.096**	
565:425	6.173	5.963	6.105	**6.080**	SEM = 0.0349 (Effect of cereal)
700:300	6.034	6.054	5.957	**6.015**	
Effect of cereal (p>0.05)	**6.109**	**6.016**	**6.110**		SEM = 0.6980 (Effect of AM:AP ratio*cereal) (p>0.05)

TABLE 2. 18. THE EFFECT OF CEREAL TYPE AND AMYLOSE:AMYLOPECTIN RATIO (AM:AP) ON VILLUS LENGTH, VILLUS THICKNESS AND THE NUMBER OF GOBLET CELLS.

5. 5. 1. Villus length (μm).

	Cereal type				
AM:AP ratio in maize starch ⇓	Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio ($p<0.001$)	
250:750	888	967	1025	**960**	SEM = 13.6 (Effect of AM:AP ratio)
385:615	787	912	938	**879**	SEM = 16.6 (Effect of cereal)
Effect of cereal ($p<0.001$)	**837**	**940**	**981**		SEM = 23.6 (Effect of AM:AP ratio*cereal) ($p>0.05$)

5. 5. 2. Villus thickness (μm).

	Cereal type				
AM:AP ratio in maize starch ⇓	Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio ($p<0.001$)	
250:750	164	122	120	**135.3**	SEM = 3.5 (Effect of AM:AP ratio)
385:615	192	161	136	**163**	SEM = 4.3 (Effect of cereal)
Effect of cereal ($p<0.001$)	**178**	**141**	**128**		SEM = 6.1 (Effect of AM:AP ratio*cereal) ($p>0.05$)

5. 5. 3. Goblet cells in villus.

	Cereal type				
AM:AP ratio in maize starch ⇓	Wheat	Long grain rice (high amylose)	Short grain rice (low amylose)	Effect of AM:AP ratio ($p<0.001$)	
250:750	74.0	72.1	71.8	**72**	SEM = 2.0 (Effect of AM:AP ratio)
385:615	97.3	82.9	85.1	**88**	SEM = 2.4 (Effect of cereal)
Effect of cereal ($p<0.05$)	**86**	**77**	**78**		SEM = 3.4 (Effect of AM:AP ratio*cereal) ($p>0.05$)

($p<0.001$). Broilers fed wheat-based diets had thicker and wider jejunal villi in comparison with chickens fed rice-based diets. Wheat-fed broilers had a greater ($p<0.05$) number of goblet cells in the jejunum.

ii). Effect of amylose:amylopectin ratio of maize starch

The different amylose:amylopectin ratios provided from the additional maize starch gave a nonlinear response in growth performance, jejunal viscosity and pH (Table 2. 18). A low weight gain and feed intake occurred when fed maize starch with the 385:615 amylose:amylopectin ratio in all three cereal diets. Digesta viscosity tended to increase ($p=0.068$) in the birds fed the 385:615 amylose:amylopectin diets. The FCR of the birds given these diets was greater ($p<0.001$) than the other amylose:amylopectin ratios although a significant interaction ($p<0.001$) was indicated because of a high FCR in the birds given the 700:300 ratio in the high amylose rice diet. The chickens fed the 385:615 amylose:amylopectin ratio had shorter and thicker jejunal villi ($p<0.01$) and an increased number of goblet cells in comparison with the birds fed diets containing starch with the 250:275 amylose:amylopectin ratio.

2. 2. 2. 6. Discussion

The lower amylose rice sample gave greater chicken growth rates and FCRs than the high amylose rice sample. This effect was opposite to that expected if a high total amylose:amylopectin ratio were nutritionally important. The improved growth

performance of the birds fed the lower amylose rice was probably a result of the increased total starch and ether extract in this sample. The evidence from this experiments suggests that the amylose:amylopectin content of the starch in rice was not a factor that affects growth performance. Panigrahi *et al.* (1992) fed two types of rice with some differences in chemical composition and did not find any differences in chicken growth performance. Bergh *et al.* (1999) did not find any significant differences in feed intake or FCR when young chickens were fed diets containing low or high-amylose barley cultivars. There were no differences ($p>0.05$) in starch, protein or fat ileal digestibility.

Wheat-based diets contained less total starch, higher overall amylose:amylopectin ratio and protein yet had higher ($p<0.001$) growth rates, feed intakes, FCRs and digesta viscosity than the rice-based diets. The feed intakes of chickens are preliminarily determined by the available energy concentration of the diet (Payne, 1967). The higher feed intakes, and poorer FCRs of the birds given the wheat-based diets was probably a result of the low total starch content giving a lower available energy concentration in the feed. Leeson *et al.* (1996) observed increased feed intake and higher FCR, when broilers were fed energy diluted diets.

The growth performance of the birds fed the four amylose:amylopectin ratios provided by maize starch was similar to that obtained before (See Section 2.2.1.5). The previous experiment showed a linear increase in growth rate and linear decrease in FCR with increasing amylose:amylopectin ratio. In the present experiment there was a non-linear response. However, apart from the relatively high growth performance of the birds given the 250:750 amylose:amylopectin ratio, the other three amylose:amylopectin ratios gave a linear response that was similar to that

obtained in the previous experiment. The interaction ($p<0.05$) between cereal type and maize amylose:amylopectin ratio was due to one value only (long-grain rice-based diet, 700:300 amylose:amylopectin ratio). This value was not consistent with the other data, and it was probably an anomaly.

The current and previous experiments have indicated that there was a growth response to the amylose:amylopectin ratio from added maize starch but that the response was independent of the total amylose:amylopectin supply from different cereals. Amylose:amylopectin ratio has been observed to affect the nutritional response of animals in other experiments. In comparison with low-amylose cornstarch, feeding high-amylose starch reduced lipid retention in the carcass without changing food intakes or weight gains of rats (Goda *et al.*, 1994). The authors suggested that high-amylose starch has a slower digestion rate than low-amylose starch and consequently may produce a lower glycaemic response and a reduced lipogenesis. The diets in the present experiment that contained added maize starch with the 385:615 amylose:amylopectin ratio decreased villus height, increased villus thickness and number of goblet cells compared to the 250:750 amylose:amylopectin ratio diet. These differences were positively related ($p<0.05$) to the differences in FCR within each of the three cereal types. Experiments with pigs (Kik *et al.*, 1990) and with broiler chickens (Viveros *et al.*, 1994) have also observed that shortening and thickening of the villi, and increasing the number of goblet cells was connected with lower growth performance. Shneeman (1982) found that decreased villus height and increased number of goblet cells in rats, could slow digestion and absorption in small intestine, because of increased mucin production. This experiment has also show that, within each of the three cereal types, the length

and the thickness of the villus were positively ($p<0.05$) related to jejunal viscosity ($r^2=0.63$ and $r^2=0.62$ respectively). Differences in viscosity may have had some effect on ileal morphometry. However, the large difference in digesta viscosity, but relatively similar growth performance, between the birds fed the wheat and rice-based diets indicated that this may not be relevant when comparing different intact cereals.

The maize starch that was added in the present experiments was chemically modified during extraction and so did not contain entire starch granules. This starch may have been more susceptible to enzyme attack within the digestive tract of the chickens. Chemically modified starch is rarely fed in practical poultry feeds, so the observed effects in the present experiments have little practical significance.

3. GENERAL DISCUSSION

Growing wheat is important for UK agriculture and more than 30% of home-grown wheat is used as animal feed. More than 500 million broiler chickens are produced in the UK alone each year and their diets are predominantly wheat-based. Relatively small variability in the nutritional value of wheat could therefore have large effects on the profitability of UK broiler enterprises. In comparison with maize, wheat has a variable nutritional quality. Metabolizable energy is a convenient measure of available energy in poultry diets and there have been a number of studies to examine the effect of chemical composition of different wheat samples on the determined ME of wheat samples. Current research has found that there is no relationship between broiler growth performance and the determined ME of the wheat samples. There is still a need to examine directly the relationship between variation in the chemical composition of different wheat samples and differences in growth performance of broilers fed the same wheat samples as part of a nutritionally complete diet.

Second, there is a need to explain the lack of relationship between determined ME and growth performance. Therefore a second objective of this project was to examine whether there were differences between different wheat cultivar samples in the efficiency of utilization of ME as a source of NE. Then to examine the relationship between differences in chemical composition of the wheat sample to any differences in the efficiency of utilization of ME.

Relationship between chemical composition of the wheat and broiler growth performance

Statistical analysis indicated that the wheat samples that had a high starch content and high amylose content within the starch were nutritionally superior. The Hagberg falling number and the content of free sugar of the samples were the other statistically significant predictors of broiler growth performance. Starch is the major energy supplier; thus it is not surprising that there was a relationship between growth performance and starch content of the wheats. The relationship between the amylose:amylopectin content of the starch and growth performance of the chickens could have a number of explanations. First, it is possible that there was a nutritional benefit in having a high total dietary supply of amylose. In comparison with amylopectin, amylose digestion may be more efficient. Second, starch granule structure is an important factor that affects broiler growth performance. Granules that have different proportion of amylose and amylopectin have different shapes and forms. The proportion of amylose and amylopectin in starch granules also affects granule integrity and its susceptibility to enzymatic and acid attack. The amylose:amylopectin relationship could therefore have been indirectly related to the different types of starch granule.

The final two experiments in the project indicated that there was a relationship between broiler growth performance and the amylose:amylopectin ratio of the additional maize starch but not to the total amylose:amylopectin ratio in the fed diets. It thus seems that starch granule structure was an important factor that affected broiler growth performance. Selection of wheat cultivars with a high proportion of

these types of starch granules could produce nutritionally superior wheat varieties in the future.

In the current study, increased HFN lead to an increase of FCR. This contradicts the results reported by other authors. However, the HFN is an empirical measurement and many other factors that affect the viscosity of the starch gel can increase the HFN of a wheat sample. It seems that in the present wheat sample set, the HFN may have not be directly important but it probably indicated previous enzymatic activity in the wheat grains. The positive relationship between the amount of free sugar and chicken FCR also supports this suggestion. Free sugar in the kernels may increase with storage because of the activity of α-amylase and other enzymes but its content is too small to be of practical nutritional importance. Hagberg falling number and content of free sugar may not be directly important for the nutritional quality of the wheat but they are probably indicators of previous enzymatic hydrolysis that occurred prior to harvest or during storage.

Relationship between the efficiency of utilization of AMEn as a source of NE and chemical and quality analysis of the wheat samples

This part of the experiment has shown that there were differences in the net energy concentration of different wheat samples that were not related to their proximate nutrient compositions. There were no consistent relationships between ME of the wheats and broiler growth performance. It indicates that the ME measurement is not the best method that could be applied to indicate the available energy concentration of different of wheat samples included in complete poultry diets. Although the net energy concentration of a wheat sample was related to its

determined AMEn, there was still unexplained variation in the efficiency of utilization of AMEn as a source of NE. A proportion of this variation was explained by differences in the water-extract viscosity of the wheat samples. Differences in water-extract viscosity probably have a multifactorial cause, but they may relate to differences in microbial fermentation in the chicken hind gut. High levels of intestinal fermentation would probably increase the heat increment of digestion and so reduce the net energy content of a feed and similarly reduce the efficiency of use of AMEn as a source of NE.

Further work that gives a greater understanding of the different efficiency of utilization of AMEn as a source of NE may enable more precise prediction of the energy availability of different wheat samples for broiler chickens.

4. GENERAL CONCLUSIONS

➤ A comparison of the nutritional quality of the twelve wheat samples showed that there were commercially important differences in weight gain, feed intake and feed conversion ratio when they were included at 650 g/kg in diets formulated for growing broiler chickens.

➤ There were significant differences in determined TMEn and NE of the wheat cultivars within the growing year, but the differences in the determined AMEn were not significant.

➤ The content of total starch and the amylose:amylopectin ratio of the starch were the main predictors of growth, feed intake and FCR. In addition Hagberg falling number was important as a third independent variable of prediction of broilers growth and feed intake. The amount of free sugar used as a third indeperndent variable improve the prediction of FCR.

➤ The efficiency of utilization of AMEn for NE varied between the wheat cultivars. The only significant relationship of the efficiency of utilization was with the water-extract viscosity of the wheat.

➤ Including an additional amount of different maize starches to wheat-based diets indicated that broiler growth performance increased due to increasing amylose:amylopectin ratios. However, changing the total dietary amylose:amylopectin ratio by including different intact cereals had no effect on growth performance.

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APPENDIX 1

MEASUREMENT OF TOTAL STARCH (A) AND AMYLOSE (B) IN WHEAT SAMPLES

200mg sample

↓

Add 5ml dimethyl sulphoxide
15 min 100°C water bath

↓

Add 15ml 95% C_2H_5OH

↓

Centrifuge 5 min, 2000 g

↓

Trough out the supernatant
Add 5ml dimethyl sulphoxide
15 min 100°C water bath

↓

Add 10ml concanavalin A (work solvent)
Drop in volumetric flask and add up to
250ml concanavalin A (work solvent)

Total starch (A)

Add 0.5ml from solution
in 4ml acetate buffer (4.5 pH)

↓

Add 0.1ml Enzyme 1
(amyloglucosidase/α-amylase solution)

↓

30 min 40°C water bath

↓

Cool at room temperature

Amylose (B)

In micro-tube add 1ml solution
Add 0.5ml Enzyme 2 (concanavalin A)

↓

Leave 55 min at room temperature

↓

Centrifuge 10 min, 20 000 g, 20°C

↓

Remove 1ml of supernatant into
3 ml acetate buffer (4.5 pH) in
15 ml centr. tubes

↓

5 min 100°C water bath
(with marble on the top)

↓

Place for 5 min in 40°C water bath

↓

Add 0.1ml Enzyme 1
(amyloglucosidase/α-amylase solution)

↓

Leave 30 min in 40°C water bath

↓

Centrifuge 5 min, 2000 g

Both

↓

Add 1ml each solution in 4 ml glucose oxidase-peroxidase reagent

↓

20 min 40°C water bath

↓

Read with 510nμ against the blank

Modified from method devised by Gibson *et al.* (1997).

APPENDIX 2

MEASUREMENT OF TOTAL (A), INSOLUBLE (B) AND SOLUBLE (A-B) NON-STARCH POLYSACCHARIDES IN WHEAT SAMPLES

Sample in duplicate (A and B)

↓

Add 2ml dimethyl sulphoxide
30 min at 100°C H_2O bath

↓

Add 8ml enzyme solution 1
10 min at 100°C H_2O bath

↓

Add 0.5ml enzyme solution 2
30 min at 50°C H_2O bath
10 min at 100°C H_2O bath

↙ ↘

Sample A
0°C ice H_2O bath
Add 0.15ml of 5M HCL
Add 40ml ac.ethanol
30 min at 0°C

↓

Centrifuge 1500 g

↓

Wash 50ml 85%ac.ethanol

Sample B
Add 40ml pH 7.0 sodium phosphate buffer
30 min at 100°C H_2O bath

↓

Centrifuge 1500 g

↓

Wash 50ml d.H_2O

↓

Centrifuge 1500 g

↓

Wash with 100% ethanol

↓

Centrifuge 1500 g

↓

Dry with acetone

↓

Add 5ml 12M H_2SO_4
30 min at 35°C

↓

Add 25ml d.H_2O
30 min at 100°C H_2O bath

↓

Add to 0.5ml sample:
0.5 dimethyl glutaric acid solution
0.1ml diluted pectinase

↓

20 min at 50°C H_2O bath

↓

Add 0.1ml of 3M NaOH
Add 1ml kit colour reagent

↓

5 min briskly boiling water

↓

Add 10ml d. H_2O

↓

Measure the absorbance at 530 nm

A - B = soluble NSP

Adapted from method devised by Englyst & Cummings (1988).

APPENDIX 3

MEASUREMENT OF RATE OF STARCH DIGESTION IN WHEAT SAMPLES

Sample in duplicate
|
Add 10 ml enzyme solution 1
|
30 min at 37°C H_2O
|
Add 5 glass marbles to each tube
Add 10 ml 0.25M sodium acetate
|
Equilibrate into H_2O for a short time
|
Remove tubes from water bath
Add 5 ml enzyme solution 2
Replace tubes in shaking water bath
|
After 10 min remove 0.5 ml of sample in 20 ml 66% ethanol
|
After 25 min remove 0.5 ml of sample in 20 ml 66% ethanol
|
After 40 min remove 0.5 ml of sample in 20 ml 66% ethanol
|
After 60 min remove 0.5 ml of sample in 20 ml 66% ethanol
|
After 120 min remove 0.5 ml of sample in 20 ml 66% ethanol
|
Centrifuge 1500g
|
Measure glucose released after 10, 25, 40, 60 and 120 minutes
at 510 nm against the reagent blank

Adapted from method devised by Englyst *et al.* (1992).

APPENDIX 4

CORRELATION COEFFICIENTS (CALCULATED AFTER REMOVING HARVEST YEAR DIFFERENCES) BETWEEN BROILERS GROWTH PERFORMANCE, DETERMINED METABOLIZABLE ENERGY, NET ENERGY AND LABORATORY MEASUREMENTS OF WHEAT CULTIVARS IN 1993 AND 1996.

	FI	WG	FCR	GE	AME	AMEn	TMEn	NEp	NE/AMEn	HFN	a-amylase	EH	1000 gw	kg/hl	DW	WV	TS	AM	AP	AM:AP	free sugar	RSD	tot NSP	sol NSP	ins NSP	Ash g	Protein g	Oil g
FI																												
WG	0.934*																											
FCR	-0.561	-0.819*																										
GE	-0.010	0.051	-0.129																									
AME	0.185	0.290	-0.404	-0.066																								
AMEn	0.122	0.226	-0.346	-0.059	0.944*																							
TMEn	-0.215	-0.027	-0.293	0.074	0.406	0.335																						
NEp	0.009	0.121	-0.272	-0.053	0.701*	0.684*	0.656*																					
NE/AMEn	-0.012	0.093	-0.240	-0.050	0.615*	0.586	0.667*	0.992*																				
HFN	-0.063	-0.020	-0.033	-0.728*	0.063	0.181	-0.003	0.348	0.358																			
a-amylase	-0.213	-0.225	0.173	0.737*	-0.013	-0.017	-0.049	-0.379	-0.420	-0.839*																		
EH	0.279	0.459	-0.588	-0.140	0.245	0.299	0.336	0.595	0.609*	0.582	-0.583																	
1000 gw	0.140	0.170	-0.179	-0.869*	0.233	0.285	0.117	0.253	0.233	0.767*	-0.796*	0.370																
kg/hl	0.129	0.077	0.035	-0.872*	-0.021	0.112	-0.190	0.020	0.004	0.789*	-0.738*	0.271	0.897*															
DW	-0.055	-0.094	0.140	0.017	-0.022	0.149	-0.171	-0.300	-0.361	0.008	0.318	-0.284	-0.010	0.218														
WV	-0.168	-0.391	0.645*	-0.470	-0.377	-0.372	-0.400	-0.555	-0.552	0.042	0.048	-0.624*	0.129	0.363	0.364													
TS	0.345	0.309	-0.172	0.546	0.494	0.435	0.152	0.374	0.339	-0.523	0.391	-0.113	-0.452	-0.516	-0.142	-0.241												
AM	0.409	0.470	-0.421	0.505	0.255	0.317	-0.165	0.275	0.251	0.017	0.062	0.420	-0.301	-0.297	0.062	-0.668*	0.312											
AP	0.259	0.207	-0.075	0.446	0.454	0.377	0.201	0.323	0.292	-0.553	0.395	-0.224	-0.399	-0.467	-0.164	-0.085	0.971*	0.076										
AM:AP	-0.057	0.026	-0.137	-0.168	-0.321	-0.227	-0.243	-0.177	-0.156	0.501	-0.338	0.405	0.223	0.285	0.166	-0.244	-0.763*	0.373	-0.895*									
free sugar	-0.200	-0.419	0.664*	0.328	-0.042	0.062	-0.113	0.045	0.040	-0.203	0.405	-0.387	-0.428	-0.282	0.225	0.258	0.401	0.140	0.385	-0.324								
RSD	0.029	0.058	-0.065	-0.571	-0.073	0.076	0.069	0.224	0.236	0.789*	-0.666*	0.694*	0.569*	0.714*	0.026	-0.099	-0.620*	0.012	-0.654*	0.607*	-0.243							
tot NSP	-0.210	-0.320	0.392	0.228	-0.399	-0.400	-0.150	-0.137	-0.082	-0.095	0.059	-0.124	-0.299	-0.220	0.085	-0.142	-0.342	0.260	-0.424	0.526	0.204	0.095						
sol NSP	-0.280	-0.431	0.533	0.265	-0.300	-0.272	-0.135	-0.158	-0.128	-0.219	0.259	-0.293	-0.359	-0.243	0.270	-0.008	-0.230	0.171	-0.284	0.350	0.383	0.013	0.944*					
ins NSP	0.122	0.196	-0.253	-0.028	-0.393	-0.473	-0.088	0.013	0.099	0.302	-0.518	0.411	0.064	-0.008	-0.469	-0.405	-0.411	0.322	-0.512	0.641*	-0.414	0.251	0.470	0.153				
Ash g	-0.245	-0.311	0.370	0.439	-0.604*	-0.453	-0.332	-0.400	-0.364	-0.075	0.341	-0.062	-0.500	-0.290	0.142	0.015	-0.210	0.311	-0.298	0.404	0.516	0.091	0.420	0.402	0.182			
Protein g	-0.091	0.021	-0.179	-0.382	-0.497	-0.561	-0.087	-0.393	-0.341	0.299	-0.355	0.123	0.246	0.319	0.035	0.266	-0.665*	-0.363	-0.607*	0.419	-0.692*	0.227	-0.025	-0.196	0.449	-0.101		
Oil g	-0.149	-0.174	0.132	-0.590	0.206	0.192	0.259	0.143	0.125	0.190	-0.332	-0.271	0.615*	0.527	0.103	0.388	-0.069	-0.678*	0.097	-0.390	-0.197	0.003	-0.307	-0.221	-0.330	-0.715*	0.108	
DM	-0.021	-0.016	0.005	-0.947*	-0.106	-0.103	-0.058	-0.102	-0.094	0.709*	-0.709*	0.168	0.859*	0.856*	0.031	0.458	-0.681*	-0.546	-0.578	0.281	-0.498	0.576	-0.262	-0.341	0.128	-0.367	0.619*	0.544

(df = 9) *p<0.05 (>0.602)

FI - feed intake (kg/bird)
WG - weight gain (kg/bird)
FCR - feed conversion ratio
GE - gross energy of the wheat (MJ/kg DM)
AME - apparent metabolizable energy of the wheat (MJ/kg DM)
AMEn - nitrogen corrected apparent metabolizable enrgy of the wheat (MJ/kg DM)
TMEn - nitrogen corrected true metabolizable energy of the wheat (MJ/kg DM)
NEp - net energy for production of the wheat (MJ/kg DM)
NEp/AMEn - efficiency of utilization of NEp as an AMEn
HFN - hagberg falling number (s)
a-amylase - aplha-amylase activity of the wheat (mEU/g DM)
EH - endosperm hardness (relative units 1-100 soft > hard)
1000 gw - thousand grain weight (g)
kg/hl - kilogram per hectolitre (bushel weight)
DW - digesta viscosity (cP)
WV - water extract viscosity (cP)
TS - total starch of the wheat (g/kg DM)
AM - amylose content of the wheat (g/kg DM)
AP - amylopectin content of the wheat (g/kg DM)
AM:AP - amylose:amylopectin ratio of the wheat
free sugar - free sugar of the wheat (g/kg DM)
RSD - in vitro rate of starch digestion of the wheat (g/100g/min)
tot NSP - total non-starch polysaccharides in the wheat (g/kg DM)
sol NSP - soluble non-starch polysaccharides in the wheat (g/kg DM)
ins NSP - insoluble non-starch polysaccharides in the wheat (g/kg DM)
Ash g - ash in the wheat (g/kg DM)
Protein g - protein in the wheat (g/kg DM)
Oil g - oil in the wheat (g/kg DM)
DM - dry matter in the wheat (g/kg)